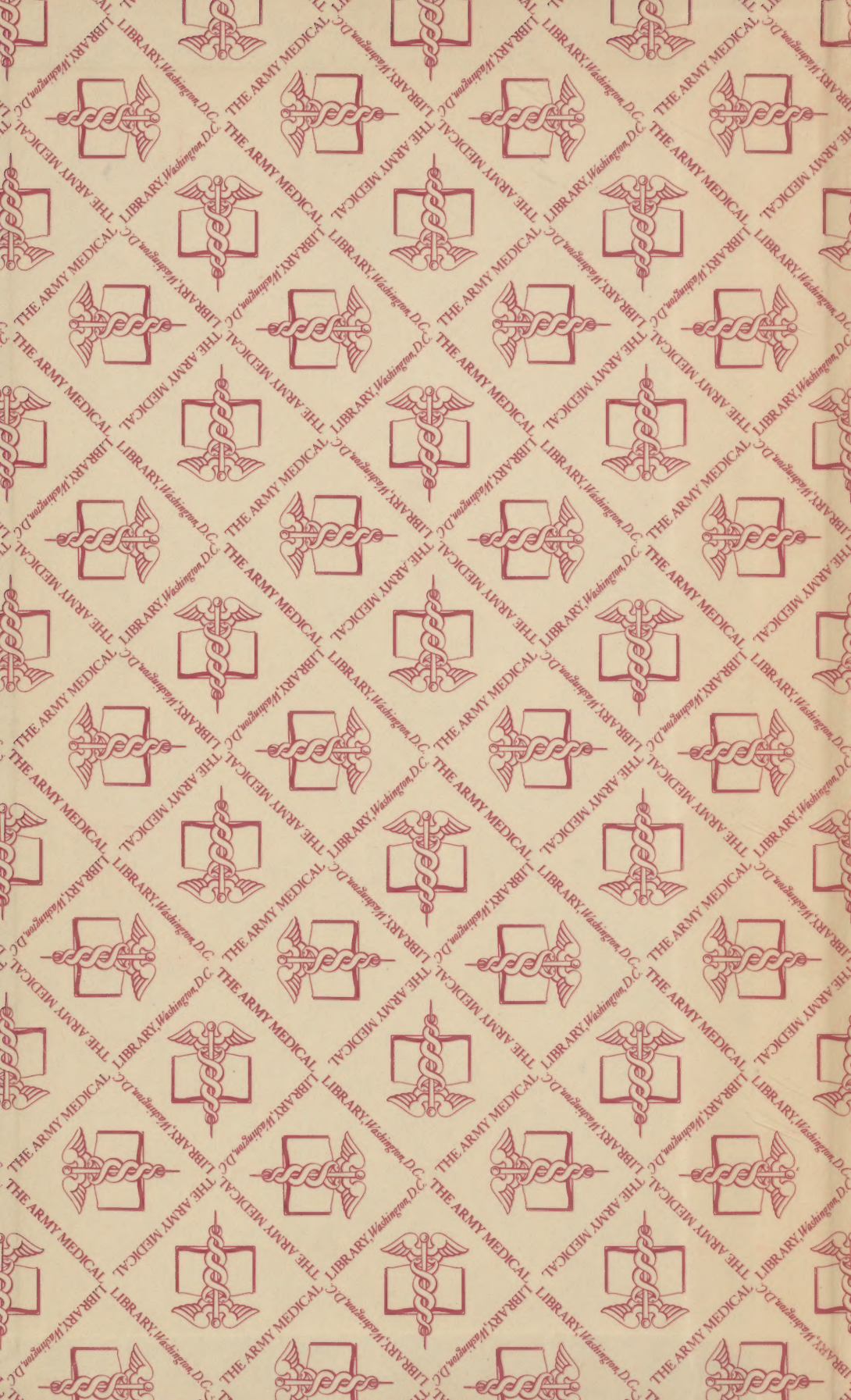
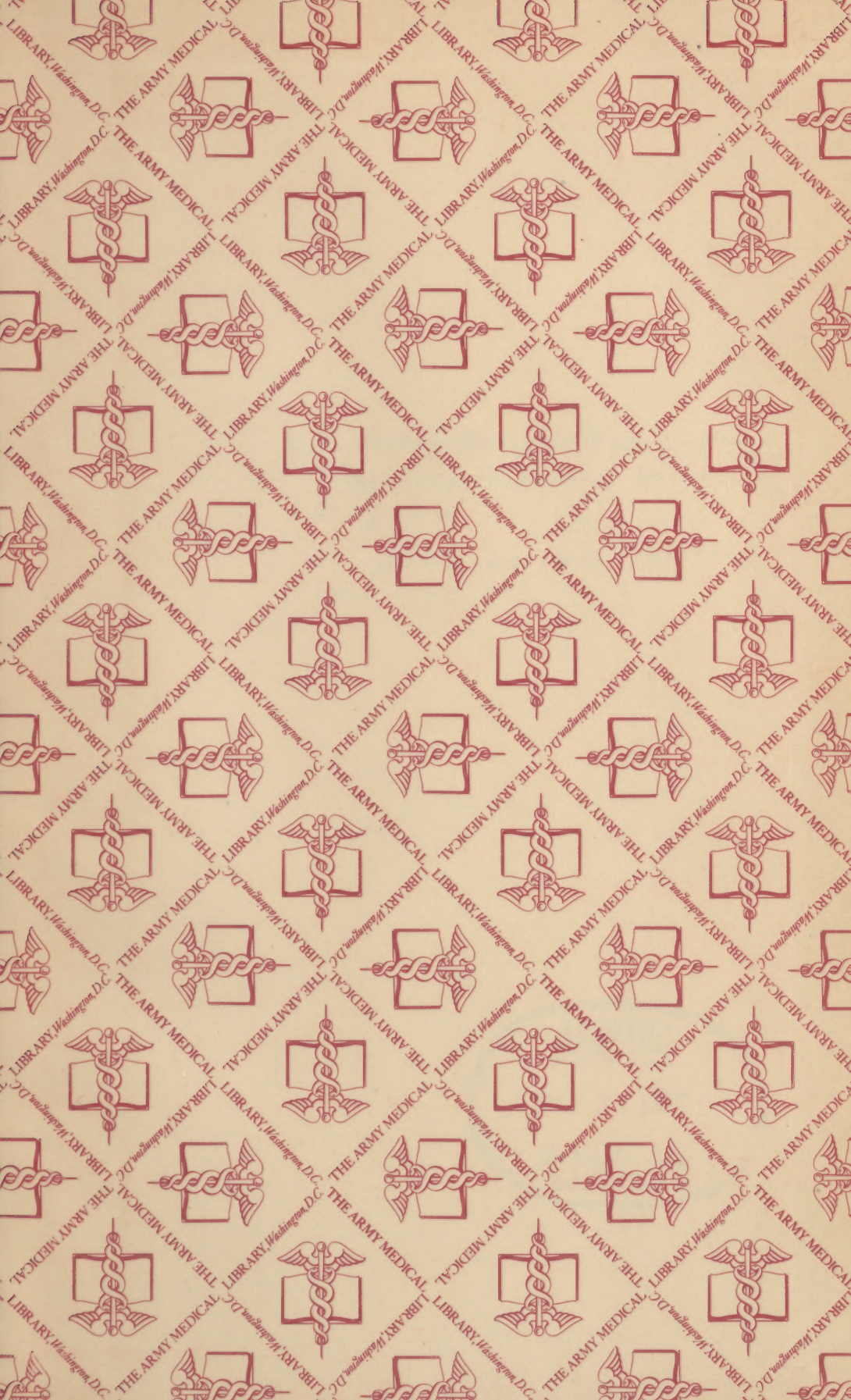

THE ARMY MEDICAL LIBRARY, Washington, D.C.


THE ARMY MEDICAL LIBRARY, Washington, D.C.

Reprinted from
THE JOURNAL
Of The
National Malaria Society
Vol. IV, No. 2 June, 1945



# STUDIES ON IMPORTED MALARIAS: 1. ABILITY OF DOMESTIC MOSQUITOES TO TRANSMIT *VIVAX* MALARIA OF FOREIGN ORIGIN*

MARTIN D. YOUNG, TRAWICK H. STUBBS, JOSEPH A. MOORE, FREDERICK C. EHRMAN, NEWTON F. HARDMAN, JOHN M. ELLIS AND ROBERT W. BURGESS
*United States Public Health Service, Columbia, South Carolina*
(Received for publication 14 November 1944)

In the fall of 1943, with the advice and approval of representatives from the Army, Navy, and certain other agencies interested in malaria, the Public Health Service in cooperation with the Army established the Imported Malaria Studies Program. This program is under the professional direction of the Malaria Investigations Office of the National Institute of Health, but is financed and staffed by the Office of Malaria Control in War Areas. Appreciation is expressed for the splendid cooperation, which made the program possible, of the Office of the Surgeon General of the Army and the staffs of the following Army General Hospitals: Letterman, Dibble, Harmon, Hammond, Moore, Oliver, Stark; also the Fort Jackson (S. C.) Station Hospital and the Naval Hospitals at Charleston, South Carolina, and Oakland, California.

The objectives of the program were concerned primarily with obtaining information related to the emerging malaria problem, as follows:

1. To determine the ability of the imported malarias to infect American anophelines and to be transmitted by them.
2. To gather information on the parasitology and other characteristics, and to distinguish, if possible, between strains.
3. To evaluate the findings and suggest their implications upon control measures.

In addition to the headquarters laboratory in Columbia, S. C., which had access to one Navy and four Army hospitals, laboratories were established in space provided by the Army in Letterman General Hospital, San Francisco, and Harmon General Hospital, Longview, Texas. (The last week in September a similar laboratory was established at Moore General Hospital, Swannanoa, N. C.). At each laboratory an insectary is maintained, with *Anopheles quadrimaculatus* Say, or *Anopheles maculipennis freeborni* Aitken, as the standard testing species. Colonies of other species are now established and several experiments have been run, but only these two species are reported here.

This report is the first of a series resulting from these studies and includes work accomplished through September 30, 1944.

*This paper was presented at the annual meetings of the National Malaria Society in St. Louis, Missouri, 14 November 1944.

## *Methods*

The principal procedures involved in the work are those which have been developed during the past few years in the Columbia laboratory*. In general, they consist of feeding mosquitoes (preferably 100 or more) on relapsing cases of malaria, dissecting them at intervals to determine rates of infection (an infection is defined as the presence of oocysts, sporozoites, or both), and feeding selected lots on neurosyphilitic patients requiring malaria therapy in order to demonstrate transmission. These procedures were pictorially presented in the February, 1944, Monthly Report of Malaria Control in War Areas.

## *Observations*

On 151 different patients 160 lots of mosquitoes have been fed. The origins of the cases studied, as nearly as could be determined, are summarized in Table 1. All of these cases were *Plasmodium vivax*. Only 3 *falciparum* cases were encountered and these are not included in this report.

Table 1.—Probable Origin of Infections Studied

| | | |
|---|---|---|
| South Pacific Area | | 136 |
| Guadalcanal | 96 | |
| New Guinea | 33 | |
| Other | 7 | |
| Mediterranean Area | | 18 |
| Europe | 9 | |
| Africa | 9 | |
| South American Area | | 6 |
| Trinidad | 6 | |
| Total Lots Fed (On 151 different patients) | | 160 |

In the Texas and California laboratories cases were more carefully selected so as to use those showing gametocytes in the peripheral blood, whereas at Columbia feedings were usually made on all cases showing parasitemia. The summaries of the lots fed as compared with the infected lots must be interpreted in light of this fact. Table 2 shows lots of mosquitoes applied and the number infected expressed in percentages. A lot was considered infected if it showed one or more infected mosquitoes.

*Burgess, R. W. and Young, M. D. Methods of Rearing and Feeding *Anopheles quadrimaculatus* Say upon Malarious Patients. Jour. Nat. Mal. Soc., 3: 241-247. 1944

From Table 2, it is interesting to observe that the rate of infection according to lots is about the same for the three widely separated areas.

TABLE 2
Number of Lots Fed and Percent Infected According to Origin of the Infections

| | Columbia (*A. quad.*) | | Texas (*A. quad.*) | | California (*A. free.*) | | Total | |
|---|---|---|---|---|---|---|---|---|
| Total | Fed. 68 | Inf. 54% | Fed. 31 | Inf. 77% | Fed. 61 | Inf. 77% | Fed. 160 | Inf. 68% |
| Pacific Area | 45 | 47% | 31 | 77% | 60 | 78% | 136 | 68% |
| Mediterranean Area | 17 | 71% | – | – | 1 | 0% | 18 | 67% |
| South America | 6 | 67% | – | – | – | – | 6 | 67% |

Table 3 shows the total numbers of mosquitoes fed, dissected, and found infected, with percentages.

Among the infected lots, the total number of infected *A. quadrimaculatus* were 959 out of 2,489 or 38.5 percent while the *A. m. freeborni* showed 1,358 infected out of 2,581 dissected, or 52.6 percent. It is also noted, however, that among the *A. quadrimaculatus* at Columbia there were 460 out of 1,072, or 42.9 percent infected, whereas in Texas there were 499 out of 1,417, or 35.2 percent infected.

TABLE 3
Summary of 160 Mosquito Infection Experiments on 151 *vivax* Malaria Patients

| | Columbia (*A. quad.*) | Texas (*A. quad.*) | California (*A. free.*) | Total |
|---|---|---|---|---|
| Total lots fed | 68 | 31 | 61 | 160 |
| Total mosquitoes fed | 5,599 | 2,847 | 8,888 | 17,334 |
| Infected lots | 37 | 24 | 47 | 108 |
| Mosquitoes fed | 3,138 | 2,389 | 7,032 | 12,559 |
| Mosquitoes dissected | 1,072 | 1,417 | 2,581 | 5,070 |
| Mosquitoes infected | 460 | 499 | 1,358 | 2,317 |
| Percent infected | 42.9 | 35.2 | 52.6 | 45.7 |

There are many factors influencing the figures given for the infected lots of mosquitoes other than the susceptibility of the mosquitoes, the most obvious being the number of mature gametocytes in the peripheral circulation. The influence of this factor, although definitely affecting these data, is not used here as a criterion in separating lots. Since the gametocyte threshold necessary for infection has not been accurately determined, and since other factors have an influence, we have separated lots simply on the basis of whether or not any infected mosquitoes were found.

Whether the difference in the infection rates of the *A. quadrimaculatus* and *A. m. freeborni* is significant has not been tested adequately. Observations are now under way to elucidate this point.

To prove the actual transmission of these malarias, infected mosquitoes were applied to neurosyphilitic patients. Arrangements were made with the various hospitals to use these malarias in the treatment of neurosyphilis. The summary in Table 4 shows the results of the attempts to transmit them to white patients. Additional attempts made on Negro patients are not included in this summary because of the possible potential difference in immunity. The Negro transmissions will be handled as separate experiments.

TABLE 4

Summary of Attempts to Transmit Imported *P. vivax* to Neurosyphilitic Patients.
Infected Mosquitoes from 32 cases were fed on 59 patients.

| | Pacific | | Mediterranean | | Total | |
|---|---|---|---|---|---|---|
| | Attempts | Successes | Attempts | Successes | | |
| Infected Mosquito Lots | | | | | | |
| *A. quad.* | 18 | 11 | 5 | 3 | 23 | 14 |
| *A. freeborni* | 9 | 7 | | | 9 | 7 |
| Total | 27 | 18 | 5 | 3 | 32 | 21 |
| Patients | | | | | | |
| *A. quad.* | 45 | 33 | 5 | 3 | 50 | 36 |
| *A. freeborni* | 9 | 7 | | | 9 | 7 |
| Total | 54 | 40 | 5 | 3 | 59 | 43 |

On a basis of comparison with American strains, the imported cases are compared with feedings on the St. Elizabeth's strain of *P. vivax*. No series of cases of indigenous malaria is available for comparison. An attempt was made along this line, but too late in the season, and only three *vivax* cases originating in the United States were tested. Table 5 shows figures for infected lots and total numbers of infected mosquitoes, comparing St. Elizabeth's *P. vivax* in *A. quadrimaculatus* to foreign *P. vivax* in *A. quadrimaculatus* and *A. m. freeborni*.

TABLE 5
Mosquito Infecting Experiments Showing
Comparison of Foreign with St. Elizabeth's strain of *P. vivax*

| | St. Elizabeth | Foreign | |
|---|---|---|---|
| | *A. quad.* | *A. quad.* | *A. freeborni* |
| Infected lots | 108 | 61 | 47 |
| Mosquitoes dissected | 2,277 | 2,489 | 2,581 |
| Mosquitoes infected | 1,128 | 959 | 1,358 |
| Percent infected | 49.5 | 38.5 | 52.6 |

## *Summary*

1. One hundred sixty (160) lots of mosquitoes were fed on 151 patients relapsing with *Plasmodium vivax* malaria of foreign origin (99 lots of *A. quadrimaculatus* on 96 patients and 61 lots of *A. m. freeborni* on 55 patients). Infections were produced in 108, or 68 percent of these lots.

2. Of 17,334 mosquitoes fed on these foreign cases, 12,559 were in the 108 infected lots. Of the latter, 5,070 were dissected and 2,317, or 45.7 percent revealed either oocysts or sporozoites or both (959 out of 2,489 *A. quadrimaculatus,* or 38.5 percent; and 1,358 out of 2,581, or 52.6 percent in *A. m. freeborni*). Of 108 lots of *A. quadrimaculatus* fed on cases of St. Elizabeth strain of *P. vivax,* 1,128, or 49.5 percent, of 2,277 dissected were infected.

3. From 32 imported malaria cases, transmission to 59 white patients was attempted. Twenty-one (21) of these strains produced infections in 43 patients.

## *Conclusions*

On the basis of the evidence so far, the following conclusions appear to be justified:

1. *Plasmodium vivax* malaria contracted by soldiers in foreign countries (South Pacific, Mediterranean, and South American areas) which relapses after their return to this country is infective to the native malaria vectors, viz., *Anopheles quadrimaculatus* Say and *Anopheles maculipennis freeborni* Aitken.

2. These mosquitoes infected by the imported *vivax* malaria can transmit the disease by biting a susceptible person.

3. Control measures are as necessary for imported malarias as for native malarias. Military personnel relapsing with imported malarias in an area where malaria vectors are present would offer possibilities for transmission to the population similar to a corresponding number of native malaria cases.

*Reprinted from*
*The Journal of the*
*National Malaria Society,*
*Vol. IV, No. 4, Dec., 1945*





# STUDIES ON IMPORTED MALARIAS: 2. ABILITY OF CALIFORNIA ANOPHELINES TO TRANSMIT MALARIAS OF FOREIGN ORIGIN AND OTHER CONSIDERATIONS[1]

JOSEPH A. MOORE, MARTIN D. YOUNG, NEWTON H. HARDMAN
and TRADWICK H. STUBBS
*United States Public Health Service, Columbia, South Carolina*[2]

(Received for publication 3 August 1945)

## *Introduction*

This report records observations made on the ability of California anophelines to transmit malarias of foreign origin. A previous report has given in general (Young, Stubbs, Moore, Ehrman, Hardman, Ellis and Burgess, 1945) the results obtained through September 30, 1944, for the several laboratories of the "Imported Malaria Studies" program. The present report will deal more in detail with the results of experiments conducted in the laboratory which was located in the Letterman General Hospital and which operated from November, 1943, to December, 1944.

## *Methods*

The usual procedure was to feed mosquitoes upon volunteer returned service men relapsing with foreign malaria. In some instances mosquitoes so infected were applied to mental patients requiring malaria therapeutically at State Hospitals. From such induced cases other feedings were made to test further the infectivity of particular infections.

At the time of application of mosquitoes, white blood cell counts were usually made, as well as routine blood and exflagellation smears.

When the work first began, an attempt was made to select patients with demonstrable gametocytes. Later this became impractical and some patients were tested without determining the gametocyte density beforehand.

After feeding, the mosquitoes were placed in a constant temperature and humidity cabinet controlled to 75°F. plus or minus 1.0° and a relative humidity of 80-90 percent, with the exception of the first few weeks during which time the usual temperature maintained was around 73° F.

[1]Contribution from the Imported Malaria Studies program of the Office of Malaria Investigations, National Institute of Health, and the office of Malaria Control in War Areas.

[2]The following hospitals made available soldiers with relapsing malaria infections: Letterman General, Hammond General, Dibble General, Oakland Naval, and U. S. Marine. The mental hospitals cooperating were the California State Hospitals at Napa and Agnew and the U. S. Veterans Hospital at Palo Alto. To these, and especially to Letterman General Hospital which also furnished laboratory quarters, we express appreciation. Also, thanks are due to the Division of Entomology of the University of California and to the California project of Malaria Control in War Areas and particularly to G. E. Washburn and R. Rosensteil, for their valuable cooperation.

While in the cabinet the mosquitoes were fed nightly by placing on top of the jars gauze strips wet with 5 percent glucose solution. These strips were removed each morning and washed. The mosquitoes were dissected 6, 8, 10, 12 and 14 days after feeding and on such other days as indicated.

Four species of California mosquitoes were used in this study, viz., *Anopheles maculipennis freeborni* Aitken, *A. m. occidentalis* (D and K), *A. psuedopunctipennis franciscanus* (McCracken) and *A. punctipennis* (Say). *A. m. freeborni* was used as the standard testing species and the others were compared with it. For the purpose of this report, the nomenclature of Aitken (1945) has been followed.

The origins of the different species used were as follows: *A. m. freeborni*—Marysville, Auburn, Riverside, and Merced, California; *A. punctipennis*—San Francisco Bay Area and Auburn, California; *A. p. franciscanus*—San Francisco Bay Area and Marysville, California; *A. m. occidentalis*—San Francisco Bay Area, California, and adjacent coastal region.

A colony of *A. m. freeborni* was established from field caught females which came principally from the vicinity of Marysville, California. The mosquitoes used for transmission came from the colony, except a few which were collected in the field for special purposes.

A "lot" of mosquitoes is defined as a group of mosquitoes of the same species fed upon a patient at one time. It usually consisted of 100 or more mosquitoes. A lot was considered infected which showed one or more mosquitoes with oocysts on the gut, sporozoites in the glands, or both.

Starting at 501, each infection was given a serial number. Symbols were designated for the various origins as follows: N. G.—New Guinea; G—Guadalcanal; N. B.—New Britain; N. Ge.—New Georgia; B—Bougainville; Si—Sicily. The origin symbol follows the serial number.

During the course of this work, about 100,000 anophelines were handled. Of these, 89,497 were reared in the insectary as follows: *A. m. freeborni* 84,668; *A. punctipennis* 2,124; *A. m. occidentalis* 2,175; and *A. p. franciscanus* 530. The rest were field caught specimens

A total of 89 feedings (lots) was made in which 11,592 (79 percent) mosquitoes fed out of 14,640 applied. Seventy-three patients (relapsing soldiers—64; neurosyphilitics—9) who had foreign malarias, were fed upon.

## *Observations*

*Origin and Infectivity of the Relapsing Malarias.* Sixty-four lots of mosquitoes (*A. m. freeborni*) were fed upon 63 returned service men relapsing with foreign *Plasmodium vivax* malaria, as shown

in Table 1.

Table 1.—Infectivity of foreign relapsing *P. vivax* to *A. m. freeborni*

| | Patients upon whom mosquitoes were fed | | Mosquitoes fed on patients | |
|---|---|---|---|---|
| Probable Origin of Infection | Number | Number Who Infected Mosquitoes | Number of Lots Fed | Number of Lots Which Became Infected |
| Guadalcanal | 26 | 24 | 26 | 24 |
| New Guinea | 18 | 10 | 19 | 10 |
| Other South Pacific* | 18 | 14 | 19 | 14 |
| ***Total South Pacific*** | **62** | **48** | **64** | **48** |
| Mediterranean | 1 | 0 | 1 | 0 |

* These include New Georgia, New Britain, Australia, Bougainville, and Indefinite.

The infectivity of the *vivax* infections to *A. m. freeborni* is shown in Table 2.

Table 2.—Feeding, dissection and infection data in 48 lots of A. m. *freeborni* infected with *P. vivax*

| | |
|---|---|
| Mosquitoes Fed | 7,444 |
| Mosquitoes Dissected | 2,711 |
| Mosquitoes Infected | 1,428 |
| Per Cent Infected | 52.67 |

The 17 lots which failed to show infections contained 2,180 mosquitoes of which 660 were dissected.

The average rate of infection for all of the mosquitoes in the total 65 lots was 42.36 per cent.

The infection rates of the forty-four infections originating from Guadalcanal and New Guinea were compared. Out of the 1,449 mosquitoes dissected which had fed upon Guadalcanal infections, 41 per cent were infected; of the 831 of those fed upon New Guinea infections, 37 per cent were infected. It appears that there is little diffence in the infectivity of the infections originating from these two areas.

In addition to the above *P. vivax* infections, there was one *P. falciparum* infection tested which apparantly originated from Guadalcanal. It showed 0.8 gametocytes per 100 white blood cells (86 per cmm.) at the time of feeding, at which time exflagellation was demonstrated.

Twenty-eight mosquitoes were dissected. One gut with two oocysts was found on the 8th day of incubation. Gland sporozoites were not found. This infection occurred even after the patient had started receiving treatment for his malaria.

Actually it is believed that a higher proportion of mosquitoes became infected than appears in the above figures because when dissections were made the mosquitoes which had not taken a blood meal and which had survived were dissected along with those which had taken blood meals. Therefore, the infectivity as expressed represents the minimum infectivity.

The fact that 74 per cent of the lots of mosquitoes fed upon the soldiers relapsing with *P. vivax* showed some infections and that 42 per cent of all mosquitoes applied became infected, indicates that under the conditions of the experiments, these foreign malarias readily infected *A. m. freeborni.*

With two exceptions, each soldier was tested only one time. It is likely that a relapsing soldier might not be infective to mosquitoes at one time and might be infective at another time in the relapse. So, some of those which did not infect mosquitoes might have done so at another trial.

Apparently it can be concluded that these relapsing foreign *vivax* malarias show a high rate of infectivity to *A. m. freeborni.*

*The Infectivity to A. m. freeborni of Seven Foreign vivax Malarias Induced in Nine Neurosyphilitics Compared to the Same Infections in the Relapsing Soldiers.* Further tests of the infectivity of these foreign malaries were made by using neurosyphilitics to whom these malaries had been transmitted. This infectivity is compared to that of the same strains in the soldiers.

The malaria induced in the neurosyphilitics was in the primary attack at the time of feeding mosquitoes. In the soldiers, infection 528 apparently was in the primary attack and the rest were relapses.

Table 3 shows the results of these experiments.

Table 3—Infectivity to *A. M. freeborni* of seven foreign *vivax* malarias inducted in nine neurosyphilitics compared to the same infections in relapsing soldiers.

| | Neurosyphilitics | | | Relapsing Soldiers | | |
|---|---|---|---|---|---|---|
| Infection Number | Gametocytes per 100 wbc | Mosquitoes Dissected | % Infected | Gametocytes per 100 wbc | Mosquitoes Dissected | % Infected |
| 506G | 10 | 71 | 94 | 6 | 69 | 93 |
| 528NG | 2 | 23 | 87 | 2 | 103 | 89 |
| 541G | 12 | 27 | 100 | 3 | 123 | 76 |
| 541G | 2 | 18 | 72 | | | |
| 543G | 16 | 24 | 88 | 2 | 66 | 65 |
| 543G | 10 | 19 | 0 | | | |
| 552NG | 4 | 36 | 83 | 3 | 152 | 24 |
| 559NG | 4 | 32 | 81 | 8 | 40 | 88 |
| 561NG | 7 | 24 | 96 | 13 | 50 | 74 |
| Totals and Averages | 7.4 | 274 | 82.9 | 5.3 | 603 | 66.3 |

All of the feedings on the neurosyphilitics were made during the primary attack when the average gamtocyte count was 40 percent higher than in the relapses of the soldiers.

There were 25 percent more infected mosquitoes in the feedings upon the neurosyphilitics than in those of the relapsing soldiers. However, with the exception of 552-NG in the soldier patient, all of the trials gave relatively high infection rates in both soldiers and neurosyphilitics.

*Infectivity of Foreign vivax Malarias to Various Species of California Mosquitoes.* As far as could be determined there are few experimental data concerning the ability of western anophelines to act as vectors for malaria and apparently there are no data on California mosquitoes. To obtain such information, experiments were run to compare *A. p. franciscanus, A. m. occidentalis,* and *A. punctipennis* to the standard testing species of *A. m. freeborni.*

The results of feeding these different species of mosquitoes simultaenously on foreign malarias are shown in Table 4.

All four species became infected. In every case where the *A. m. freeborni* became infected, the other species tried at the same time also became infected. When *A. m. freeborni* did not become infected, neither did the other species.

Inasmuch as each of the species differed in the proportion of those applied that bit, a comparison of the percentages of infection might not be representative of the susceptibility of that species to malaria. A comparison of the number of oocysts on the gut might be a better measure.

Such a comparison (Table 4) showed that the different species involved in a single feeding had about the same intensity of gut infection. Furthermore, sporozoites were found in all of the species tried.

A single attempt was made to transmit the infection to a neurosyphilitic by each *A. m. occidentalis* and *A. punctipennis.* Both resulted in infections. No attempt to transmit was made with *A. p. franciscanus.*

*Length of Sporogonous Cycle of Foreign P. vivax in A. m. freeborni.* Out of 57 lots of mosquitoes infected upon relapsing soldiers and neurosyphilitics, 29 were followed by daily dissections to obtain the length of the sporogonous cycle in *A. m. freeborni* by determining the first day of appearance of sporozoites in the glands. These data are presented in Table 5.

Table 5.—Length of sporogonous cycle of foreign *P. vivax* in *A. m. freeborni*

| Donor | Incubation Temperatures | First Day of Sporozoites In Glands in 29 Lots of Mosquitoes | | | | | | Total Lots | Average Days |
|---|---|---|---|---|---|---|---|---|---|
| | | 9 | 10 | 11 | 12 | 13 | 14 | | |
| Soldiers | 75° F. | 5 | 8 | 4 | 2 | 0 | 0 | 19 | 10.16 |
| Soldiers | 73° F. | 2 | 3 | 0 | 0 | 0 | 1 | 6 | 10.33 |
| Neurosyphilitics | 75° F. | 1 | 3 | 0 | 0 | 0 | 0 | 4 | 9.75 |
| TOTAL | | 8 | 14 | 4 | 2 | 0 | 1 | 29 | 10.07 |

Table 4.—Comparison of infectivity of foreign *vivax* malaria to various species of California mosquitoes

| Infections | A. m. freeborni | | | A. punctipennis | | | A. m. occidentalis | | | A.p. franciscanus | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Diss. | Inf. | Intensity oocysts | Diss. | Inf. | Intensity oocysts | Diss. | Inf. | Intensity oocysts | Diss. | Inf. | Intensity oocysts |
| 503G | 44 | 18 | 2.2 | 31 | 4 | 1.5 | | | | | | |
| 541G* | 18 | 13 | 1.0 | 19 | 8 | 1.0 | | | | | | |
| 543G* | 24 | 21 | 3.2 | 24 | 14T | 3.8 | | | | | | |
| 543G* | 19 | 0 | 0.0 | 15 | 0 | 0.0 | 8 | 0 | 0.0 | | | |
| 552NG* | 36 | 30 | 1.1 | | | | | | | 9 | 4 | 1.0 |
| 560NG | 74 | 48 | 1.6 | | | | | | | 14 | 8 | 1.3 |
| 559NG* | 32 | 26 | 1.6 | | | | 24 | 19 | 1.1 | | | |
| 561NG* | 24 | 23 | 2.7 | | | | 27 | 17T | 2.8 | | | |

Legend:
In determining the average intensity of the gut infections the number of oocysts on the guts were expressed as follows: 1–9 oocysts = +; 10–24= ++; 25–99 = +++; 100+ = ++++. In the above table these intensity groups were averaged and expressed in numbers, i.e., the average of + and ++ is expressed as 1.5
* — Indicates feeding made in neurosyphilitics in whom the foreign malarias had been induced. The other feedings were on the relapsing returned soldiers.
T — Transmission to another patient tried and successful.

The above table indicates a rather rapid development of the infection in the *A. m. freeborni*. Fourteen other infected lots of this species dissected at 2 day intervals were not included in the above table but the length of the sporogonous cycle appears to be similar.

The shortest sporogonous cycle noted was from Infection 506-G which had been transmitted to a neurosyphilitic. Sporozoites were found in the glands on the 8th day after incubation at 75° F. This was not included in the above table as dissections had not been done daily.

Three experiments were run comparing *A. m. occidentalis, A. p. francisanus,* and *A. punctipennis* with *A. m. freeborni* as to length of sporogonous cycle. In each instance the two species involved were fed on the patient at the same time and kept under identical conditions at 75° F.

In the two experiments testing *A. punctipennis* and *A. m. occidentalis* against *A. m. freeborni,* all showed gland sporozoites on the tenth day. In the third experiment, *A. p. franciscanus* showed gland sporozoites on the tenth day. However, the control *A. m. freeborni* were not dissected on the tenth day but dissection on the ninth day had shown nearly mature oocysts. This, in addition to the average findings of the cycle (Table 5), indicates that the development in *A. m. freeborni* is 10 days and that sporozoites probably would have been found in the glands on that day had dissections been done.

These results indicate that the length of the sporogonous cycle is the same (10 days) in the four mosquito species tested. This supports the data in the Table 4 indicating that the susceptibility of these four species to foreign *vivax* is similar.

*Transmission of Infections to Man By Mosquitoes.* While the presence of normal sporozoites in the glands is presumptive proof of transmissibility of malaria, certain lots of infected mosquitoes were applied to neurosyphilitics for final proof.

The data on the attempts are shown in Table 6. Also included in this table are the attempts to infect mosquitoes from the induced cases.

As seen in Table 6, attempts were made to transmit by mosquitoes 19 infections to 23 persons in 24 trials. Seventeen different strains were transmitted successfully to 21 patients in 22 attempts.

Twelve infections were successfully transmitted by mosquitoes to each of 12 patients, and 5 infections transmitted to 2 patients each. One patient (R. P.) became infected with both 506G and 541G . One patient (W. M.) was infected by blood transfer.

Table 6.—Attempts to transmit foreign *vivax* malaria to man by mosquitoes

| Transmission Attempts | Strain No. | Patient Name | Race | Days Pp. | Days Inc. | Resulting Infections in Man: Mosquitoes Subsequently Fed Species | Infected | Remarks |
|---|---|---|---|---|---|---|---|---|
| 1 | 506G | M. V. | N | 14 | 12 | F | + | |
| 2 | 506G | R. P. | W | 14 | 13 | | | |
| 3 | 510NB | A. P. | W | 15 | 15 | | | |
| 4 | 513G | H. G. | N | 11 | 14 | | | |
| 5 | 516G | T. C. | C | 13 | 13 | | | |
| 6 | 518NG | F. R. | N | | | | | Had Malaria Before |
| 7 | 519B | L. D. | N | | | | | Had Malaria Before |
| 8 | 520NG | R. H. | W | 12 | 16 | | | |
| 9 | 523G | W. M. | W | 12 | 10 | | | |
| 10 | 524G | J. Z. | W | 12 | 10 | | | |
| 11 | 528NG | T. B. | W | 13 | 12 | | | |
| 12 | 528NG | G. W. | W | 13 | 12 | F | + | |
| 13 | 534NG | W. K. | W | 11 | 11 | | | |
| 14 | 535NG | V. S. | W | 16 | 13 | | | |
| 15 | 541G | R. F. B. | W | 10 | 10 | F | + | Transmitted to R. P. |
| 16 | 541 | R. P. | W | 16 | 16 | F, P | +,+ | |
| 17 | 542G | H. B. | W | 29 | 19 | | | |
| 18 | 543G | J. M. | W | 13 | 14 | | | Blood to W. M. |
| | 543 | W. M. | W | Blood Transfer from J.M. | | F. P. | +,+ | Transmitted to P. K. |
| 19 | 543 | P. K. | W | 19 | 11 | F, P, O, | —,—,— | |
| 20 | 552NG | J. P. | W | 12 | 14 | F, $P_F$ | +,+ | |
| 21 | 559NG | G. R. | W | 12 | 12 | F, O | +,+ | |
| 22 | 560NG | E. P. | W | 20 | 18 | | | |
| 23 | 561NG | E. R. | W | 10 | 11 | F, O | +,+ | Transmitted to S. L. |
| 24 | 561 | S. L. | W | 12 | 11 | | | |
| Totals 24 | 19 | 23 | | 309 | 287 | 9 | 8 | |
| Averages | | | | 14.05 | 13.05 | | | |

Legend: F = *A. m. freeborni;* P = *A. punctipennis;* O = *A. m. occidentalis;* $P_F$ = *A. p. franciscanus;* N = Negro; W = White; C = Chinese; — = No infection + = Infection; Pp = Prepatent period of parasites; Inc = Incubation period of symptoms.
Transmission of all by *A. m. freeborni* except attempt # 24 which was by *A. m. occidentalis* and # 19 by *A. punctipennis.*

Two transmission attempts failed. The recipients were Negroes who had had malaria therapeutically before. It is not possible to state whether either the race of the patient, the previous malaria, or both, operated against the infection developing. It is interesting that the other two Negros involved (M. V. and H. G.) developed malaria producing at least 16 and 15 paroxysms respectively. In these two cases, the race seemed to exert no influence.

On the other hand, one white patient (R. P.) developed infections after each of two transmission attempts (506G and 541G). With the first infection (506G) he had 12 paroxysms. He was given the second malaria (541G) five months after the first one. The second infection was not followed long enough to determine the number of paroxysms produced. While it is not known how much immunity the first infection exerted against the second infection in the length of the clinical manifestations of the latter, it is seen that the immunity was not great enough to prevent the second infection from developing within the normal range (16 days).

*Development of P. vivax Infections in A. m. freeborni at Outside Temperatures.* Experiments were set up to determine how these foreign malarias would develop at outdoor temperatures. Both field caught and laboratory-bred *A. m. freeborni* were employed. In each experiment, one-half of the mosquitoes which had been applied to the malarious patient were put into a constant temperature cabinet maintained at 75° F. in the San Francisco laboratory. The rest of the mosquitoes were put under shelters at outdoor temperature. They were handled in the routine manner otherwise.

Temperature readings were taken from the official weather reports. The San Francisco readings were taken at the weather bureau several miles away from the barn where mosquitoes were kept. Sacramento readings (nearest weather station to Marysville) were taken about 50 miles from Marysville but were in the same general climate. Readings taken at Marysville during this time were generally the same as shown by the weather bureau records at Sacramento.

The temperature readings at San Francisco and at Sacramento are shown in Table 7.

Table 7.—Temperature readings during outdoor experiments

| | Sacramento, Calif. | | San Francisco | |
|---|---|---|---|---|
| Temperatures ° F. | 9/26 - 10/11 | 10/4 - 10/19 | 9/20 - 10/5 | 10/2 - 10/21 |
| Maximum Range | 99 -72 | 86 -72 | 74 - 60 | 76 - 58 |
| Maximum Average | 81 | 78 | 68 | 66 |
| Minimum Range | 59 -51 | 54 -50 | 58 - 50 | 57 - 50 |
| Minimum Average | 53 | 52 | 53 | 53 |
| Mean Range | 80 -62 | 70 -62 | 64 - 56 | 64 - 54 |
| Mean Average | 67 | 65 | 61 | 59 |

The first experiment was with part of the mosquitoes being kept under a porch in Maryville. This experiment was started on September 26th and ended October 11, 1944. The strain used was 552-NG which had been induced in a neurosyphilitic.

Of those kept entirely in Marysville, 12 out of the 16 mosquitoes showed infections whereas out of those kept in the laboratory 30 out of 36 were infected.

The mosquitoes kept in the laboratory at 75° F. showed gland sporozoites on the 11th day. At Marysville, infections developed more slowly. Oocysts on the ninth day were about the same size as the fifth day oocysts incubated at 75° F. The longest kept at Marysville were for 15 days at which time they showed oocysts ranging from half grown to almost mature. The number of the oocysts was about the same under each condition. Some of the mosquitoes were kept at Marysville for different intervals and then brought into the laboratory. These developed oocysts and gland sporozoites but the appearance of the latter was delayed considerably over those kept entirely in the laboratory at 75° F.

A second experiment was run starting October 4, 1944, at Marysville under the same conditions using Infection 541-G, which had been induced in a neurosyphilitic. Mosquitoes dissected on the 7th day showed no infection while oocysts were found on the fifth and sixth day in those kept at 75° F. On the seventh day, some mosquitoes kept outside at Marysville were put at 75° F. and on the ninth day these showed early oocysts. Others kept at Marysville and dissected on the fifteenth day showed only young oocysts about the size of the 6-7 day oocysts kept at 75° F. The controls kept at 75° F. showed gland sporozoites on the 10th day.

These results indicate that infections can occur in mosquitoes in the vicinity of Marysville under the conditions experienced, but develop more slowly than those kept at 75° F. It is to be expected that gland sporozoites would have developed if the mosquitoes had been left there long enough. During the warmer seasons it is to be expected that the infections would mature faster.

Two experiments were run at San Francisco with one half of the mosquitoes kept in a barn in the city and the others at 75° F. in the laboratory.

The first of these was with 564-NG starting September 20, 1944. Between the fifth and 15th day of development dissections were made. No infections were found in 38 dissections of the mosquitoes kept in the barn; of those kept at 75° F. 22 were dissected and all were infected. In the latter the infection was heavier than average and sporozoites were found in the glands on the ninth day.

The question then arose whether the infections were sterilized or latent in the mosquitoes kept in the barn. To elucidate this point

another experiment was run in which mosquitoes were placed in the barn for varying lengths of time and then put at 75° F. The results are shown in Table 8.

**Table 8.—The development of *P. vivax* infections in *A. m. freeborni* under different conditions of temperature in San Francisco (Oct. 2, 1944). Strain 559.**

| Days Kept | | Mosquitoes | | |
|---|---|---|---|---|
| In Barn | Then At 75° F. | Dissected | Infected | Remarks |
| 7 | 0 | 8 | 0 | |
| 8 | 0 | 8 | 0 | |
| 9 | 0 | 4 | 0 | |
| 11 | 0 | 7 | 0 | |
| 12 | 0 | 11 | 0 | |
| 14 | 0 | 7 | 0 | |
| 17 | 0 | 3 | 0 | |
| 5 | 2 | 4 | 0 | |
| 5 | 4 | 3 | 3 | Small early oocysts |
| 7 | 10 | 3 | 2 | Sporoluating oocysts |
| 9 | 2 | 8 | 5 | Small early oocysts |
| 19 | 7 | 4 | 3 | Oocysts almost mature |
| 0 | 12 | 32 | 26 | Controls — developed gland sporozoites in 11 days |

Five other mosquitoes which were incubated at 75° F. for 5 days and then put in the barn showed very little progression in development of the infection 11 and 12 days later.

Another experiment was run beginning October 6th using 561—NG in which the glands had sporozoites on the 10th day at 75° F. Some of these mosquitoes were removed after 48 hours from the 75° F. temperature and placed in the barn. The oocysts showed little development after being in the barn for as long as 8 days.

These experiments were run during what is ordinarily the warmest part of the year in San Francisco. According to the comparative data of the San Francisco weather bureau, September has been the warmest month for a 70 year period, with an average of 61.5° F. and October was the next warmest with 60.8° F.

From the above results, it seems evident that under the conditions of the experiment, infections would not develop to maturity in mosquitoes at the San Francisco temperatures.

Thus, even if mosquito carriers were present in the city an outbreak of malaria would not be expected if mosquitoes rested outdoors.

Temperature ranges shown above did not sterilize the mosquitoes even when they were kept outside for as long as 19 days. The infections subsequently developed when the mosquitoes were taken to 75° F. Thus, there is a chance of the mosquitoes biting a person and resting in a warm place, such as a house, and developing infections. However, this would not be expected ordinarily.

Furthermore, there appear to be few, if any, *A. m. freeborni* in the city of San Francisco.

So, the possibility of outbreaks of malaria in San Francisco seem very remote and would occur only under unusual conditions and probably would not be widespread.

*Relationship of P. vivax Gametocytes to Infection in A. m. freeborni.* In all cases, gametocytes were counted and expressed as the number per 100 white blood cells. In 31 instances, white blood cell counts were made at the time of feeding the mosquitoes and in these the number of gametocytes per cmm. of blood was calculated also. When a graph was plotted showing the percentage of infection in the mosquitoes against the number of gametocytes per 100 w.b.c. and against the number of gametocytes per cmm. of blood, it was found that the curves of these two methods of counting correlated closely. The white blood cell counts in 31 soldiers averaged about 6000 per cmm.

The relationship of the mosquitoes infected to the number of gametocytes is presented in Table 9.

Table 9.—Relationship of *P. vivax* gametocytes per 100 wbc to *A. m.* freeborni infected in 65 feedings of relapsing foreign *P. vivax*.

| Gametocytes per 100 WBC | Mosquito Lots | | Mosquitoes | | |
|---|---|---|---|---|---|
| | Fed | Infected | Dissected | Infected | % Infected |
| Less than 1 | 9 | 2 | 419 | 41 | 9.8 |
| 1 — 5 * | 41 | 33 | 2082 | 859 | 41.3 |
| 6 — 10 * | 7 | 7 | 339 | 250 | 73.8 |
| 11 — 15 * | 7 | 6 | 484 | 278 | 57.5 |
| 32 | 1 | 0 | 47 | 0 | 0.0 |
| Averages & Totals 4.5 | 65 | 48 | 3371 | 1428 | 42.36 |

* In the three groups including the range of gametocytes from 1 to 15 per 100 white blood cells, the average infection of the mosquitoes was 47.8 percent.

It is apparent from the data that a sharp rise in infections came when one or more than one gametocytes per 100 w. b. c. (60 per cmm.) were present. The most infective range (73.8 percent) was when from 6 to 10 were present.

The highest gametocyte count encountered was in 545-NG which showed 32 gametocytes per 100 w.b.c. (1440 per cmm). The proportion of males to females was about 1:10 or less. However, this discrepancy in sexes does not explain why it did not infect, as there were still enough males to produce a good infection as shown by other cases. Fourteen days later mosquitos were fed on the same patient with a gametocyte count of 5 per 100 w.b.c. (4 females, 1 male). No infections were found out of 40 dissections.

No reason for such a high gametocyte count failing to infect can be offered.

Conversely, two lots of mosquitoes became infected when fed on patients (558-G and 552-NG) showing no gametocytes per 100 w.b.c. One of these (552-NG) showed 30 percent of 105 mosquitoes infected; the other (558-G) showed 8 percent of 66 mosquitoes infected.

Oddly enough, 552-NG had not produced infections in mosquitoes 14 days earlier with 5 gametocytes per 100 w.b.c. (3 males, 2 females).

From the above, it appears that the following conclusion can be drawn. Foreign relapsing *P. vivax* is not always infective to *A. m. freeborni* in direct proportion to the number of gametocytes present. Generally, however, infections are most likely to be produced when there are between 1 and 15 gametocytes per 100 w.b.c. (60-900 per cmm.) with the highest infectivity (73.8 percent) within the range of 6-10 gametocytes per 100 w.b.c. (360-600 per cmm.).

*Relationship of Exflagellation of P. vivax Gametocytes to Infection in the Mosquito.* Exflagellation smears were made at the time of feeding as an aid in demonstrating the maturity of the male gametocytes. In general the method used was similar to Shute's technique as described by James (1934), except that slides remained in the moist chamber usually for 5, 10, 15, and 20 minute periods and some for 25 minutes. Both partial and complete exflagellation were recorded as exflagellation. The relationship of exflagelation to infection in the mosquito in 59 trials is set forth in Table 10.

Table 10.—Relationship of exflagellation of *P. vivax* gametocytes to subsequent infection in *A. m. freeborni*.

| Exflagellation | Mosquitoes Infected | Number Trials |
|---|---|---|
| + | + | 22 |
| + | − | 4 |
| − | + | 24 |
| − | − | 9 |

+ = Positive; − = Negative.

When exflagellation was demonstrated, infection in the mosquitoes usually occurred also (22 out of 26). This is to be expected. However it is further seen that infection occurred in a high proportion of times when no exflagellation was demonstrated (24 out of 33). One suspects that this might be a fault of the technique.

*Relationship of Number of Malaria Attacks to Number of Gametocytes Produced and Infection of Lots of Mosquitoes.* When mosquitoes were fed upon a relapsing soldier, an effort was made to determine how many attacks of malaria the patient had experienced from that infection. The first attack of malaria regardless of its relationship to suppressive drugs was designated as the primary attack. In many instances, the primary attack had been delayed considerably due to suppressive drugs. The next attack was designated as the first relapse and subsequent relapses were numbered consecutively.

After determining the relationship of gametocytes to infection of the mosquito (Table 9), the question arises about the appearance of gametocytes in the relapses and if present, whether they produce infections in the mosquitoes. Some workers believe that gametocytes become rarer as relapses occur.

The data on 64 cases are shown below in Table 11.

From Table 11 under "Gametocyte Counts", it is evident that gametocytes were produced as long as the relapses occurred. Although the sample of 64 trials was too small to indicate accurately whether fewer gametocytes were produced as relapses progressed, such a trend is not too evident from the above data.

Assuming that the gametocytes produced in the later relapses are as viable as those produced earlier, according to the interpretation of Table 9 it appears that sufficient numbers of gametocytes were produced in the later relapses to infect mosquitoes. Upon examining "Mosquito Feedings" in Table 11, it is seen that mosquitoes were infected even through the 17th relapse (18th attack). The failure of infection on the 20th relapse (21st attack) apparently is not indicative as the gametocytes were rare and also because experience in other laboratories indicates infection in even later relapses.

On the basis of the data given above, it seems reasonable to expect that some of the patients with foreign *P. vivax* will infect mosquitoes as long as relapses occur. The gametocytes appearing in the later relapses appear to be as infective as those produced in the earlier attacks.

*Intensity of the P. vivax infections in A. m. freeborni.* The number of oocysts per gut were counted or in heavy infections estimated, and recorded under the following intensity groupings: 1 — 9, +; 10 — 24, ++; 25 — 99, +++; 100+, ++++. Sporozoites were grouped as follows: 1 — 9, +; 10 — 99, ++; 100 — 999, +++; 1000+, ++++.

In 33 lots, both gut oocysts and gland sporozoites were found. The distribution of the infected mosquitoes in these lots according to the above groupings is shown in Table 12. The gut infections up to the first day of gland sporozoites and the gland sporozoites on or after the eleventh day were tabulated.

Table 12.—Intensity of infection of foreign *P. vivax* in *A. m. freeborni*

| | Number of Specimens Under Each Intensity Grouping | | | | |
|---|---|---|---|---|---|
| | + | ++ | +++ | ++++ | Total |
| Guts with Oocysts | 373 | 183 | 101 | 37 | 694 |
| Glands with Sporozoites | 122 | 138 | 127 | 67 | 454 |

Table 11.—Relationship of number of malaria attacks (*P. vivax*) to number of gametocytes and infection of lots of mosquitoes

| No. Attacks | P | Relapse Number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 17 | 20 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gametocyte Counts | | | | | | | | | | | | | | | | | | | |
| Trials | 6 | 11 | 6 | 4 | 3 | 3 | 3 | 3 | 5 | 5 | 2 | 3 | 3 | 1 | 1 | 3 | 1 | 1 | 64 |
| Av. per 100 wbc | 3.8 | 4.6 | 2.0 | 11.1 | 1.3 | 3.0 | 8.3 | 4.0 | 6.8 | 4.8 | 7.5 | 4.3 | 2.1 | 1.0 | 4.0 | 4.0 | 2.0 | 1.0 | 4.5 |
| Mosquito Feedings | | | | | | | | | | | | | | | | | | | |
| Infected | 4 | 10 | 5 | 2 | 2 | 3 | 3 | 2 | 3 | 5 | 1 | 2 | 1 | 1 | 1 | 2 | 1 | 0 | 48 |
| Not Infected | 2 | 1 | 1 | 2 | 1 | 0 | 0 | 1 | 2 | 0 | 1 | 1 | 2 | 0 | 0 | 1 | 0 | 1 | 16 |

P = Primary Infection

Table 13.—Unusually heavy infection in *A. m. freeborni* showing intensity of oocysts *P. vivax* of foreign origin

| Infection | Donor | Relapse Number | Gametocytes per 100 wbc | Gametocytes per cmm. | Guts Dissected | Guts Infected | Range of oocysts per gut and number of guts in each range 1-50 | 50+ | 100+ | 200+ | 300+ | 400+ | 500+ | 800+ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 506G | S | 7 | 6 | 462 | 33 | 28 | 15 | 1 | 5 | 1 | 4 | | 2 | |
| 506G | N | P | 10 | 1029 | 27 | 26 | 4 | 4 | 8 | 3 | 3 | | 3 | 1 |
| 524G | S | 6 | 11 | 739 | 23 | 23 | 5 | 7 | 8 | 3 | | | | |
| 505G | S | 8 | 9 | 468 | 32 | 25 | 7 | 7 | 5 | 7 | 4 | 1 | 1 | |
| 543G | N | P | 16 | 806 | 16 | 13 | @ | @ | 10@A | | | | | |

Dissections made from 5th day up through last day before sporozoites found in glands.
@ Actual oocysts counts not made. Reported as +++ (25-99, or ++++ (100 and above).
S Relapsing soldier.
N Neurosyphilitic in whom infection had been induced.
A Also *A. punctipennis* showed 6 guts with over 100 oocysts.
P Primary attack.

From these data, it is apparent that most (80 per cent) of the infected guts had between 1 and 24 oocysts and that most (85 per cent) of the glands contained between 1 and 100 sporozoites.

In 5 cases, unusually heavy infection were seen. The origin of all of these infections was probably Guadalcanal.

The data on these infections are shown in Table 13.

About 500 oocysts are about as many as we can find reported for any mosquito. Thus, the five infections are noteworthy.

The intensity of sporozoites in the glands was correspondingly high in the above infected mosquitoes.

The gametocyte counts for these five infections averaged 10.5 per 100 w.b.c. as compared to the average of 4.5 per 100 w.b.c. for the 65 total counts made. This increase in gametocytes would not seem to be enough to account for such heavy infections. Gametocyte counts higher than the above did not produce correspondingly heavy infections.

The above data suggest that these particular infections are more infective to mosquitoes than usual. Other infections also apparently originating from Guadalcanal did not show such heavy infections. However, the very fact that such heavy infections were produced indicates that some Guadalcanal infections might be more infective than others.

*Mosquito Infection after Therapy.* In 4 instances mosquitoes became infected when applied after malaria treatment had begun. Three of these were *P. vivax* and one *P. falciparum.*

Table 14.—Mosquitoes infected after patient had received treatment

| Infection Number | Mosquitoes Dissected | Mosquitoes Infected | Drug | Amount Given Before Biting |
|---|---|---|---|---|
| V-557-NG | 54 | 48 | Quinine Sulfate | 15 grains day before |
| V-543G | 66 | 43 | Quinine Sulfate | 45 grains i.v. within 24 hours before feeding |
| V-507-G | 20 | 4 | Atabrine* | 0.4 gram 2 hours before |
| F-512-G | 28 | 1 | Quinine Hydrochloride | 7.5 grains i.v.,b.i.d./3 days |

* - Blood level of 50 gammas at time of feeding
v - *P. vivax*
f - *P. falciparum*

This shows that the foreign malarias can infect mosquitoes after treatment has been started. Such results are similar to experiences in the Columbia, South Carolina, laboratory and to that of others where it had been observed that American malarias may infect mosquitoes following some treatment.

*Comparison of the Susceptibility of Field Caught and Laboratory Reared A. m. freeborni.* Two experiments were run to determine if field-caught adults and insectory-reared (6th generation) adults showed any differences in susceptibility to these foreign malaries.

In each experiment, one-half each of the field and laboratory mosquitoes were kept at 75° F. and one-half under outside conditions at Marysville such as described above. The malaria infections involved were, No. 559 and No. 561, both from New Guinea, which had been induced in neurosyphilitics.

Of the laboratory-reared mosquitoes, out of 64 dissections 40 showed infections; of the field caught specimens, out of 75 dissections, 30 showed infections.

The results indicated definitely that both the laboratory and field-caught mosquitoes were susceptible to foreign malarias. The data are not sufficient to draw final conclusions as to the relative susceptibility of field-caught against laboratory-reared mosquitoes.

*Characteristics of Foreign P. vivax Induced in Neurosyphilitics.* Slides were made and temperatures were taken at irregular times on the induced cases due to shortage of ward help at the mental hospitals. However, even with such irregular tests, the incubation and prepatent periods as determined are interesting.

The prepatent periods ranged from 10 to 29 (with only one above 20 days) averaging 14.05 days (Table 6). The incubation periods ranged from 10 to 19 days, averaging 13.05 days.

These averages represent the maximum as it is certain that in some cases had slides been made and temperatures taken daily, the observed periods would have been shortened. Even so, the prepatent and incubation periods are relatively short as compared to some of our indigenous "strains" of malaria. Unpublished data from 35 patients with *P. vivax* (St. Elizabeth strain) indicate that the prepatent period is about 15 days and the incubation period about 16 days.

Most of the induced cases showed a quotidian rather than a tertian fever periodicity. However, the use of thio-bismol when one brood of parasites were one-half grown changed the periodicity of the fevers to tertian. This is similar to the reaction of thio-bismol on the St. Elizabeth strain of *P. vivax* which is widely used in this country for the treatment of neurosyphilis (Young, McLendon and Smarr, 1943).

In the infections showing tertain periodicity of fevers, the fevers did not come at 48-hour intervals but at shorter intervals. This is in conformity to observations previously reported (Young, 1944) which showed that three strains of *P. vivax* studied had a fever periodicity of less than 48 hours.

The fever durations, fever peaks, and parasite densities seem to be in the range of that usually found with St. Elizabeth's strain (Coatney and Young, 1942.)

No outstanding differences in the course of the infections were noted.

### *Discussion*

*A. m. freeborni.* No natural or experimental infection rates have been found for *A. m. freeborni* either for California or Oregon, where for years it has been indicted as the principal vector. Two records were found for New Mexico. Barber, Komp and Hayne (1929) found 0.3 percent of 669 wild caught *A. maculipennis* infected in northern New Mexico. They also experimentally infected them with *P. vivax* but gave no figures except that *A. maculipennis* showed more infections than *A. pseudopunctipennis.* Barber and Forbich (1933) in New Mexico found 1.4 percent out of 868 wild-caught infected. Simmons and Aitken (1942) give these as the only infection rates known for *A. m. freeborni.* There appear to be no data on *P. falciparum* or *P. malariae.*

The susceptibility of *A. m. freeborni* to foreign *vivax* malaria appears to be high. Under laboratory conditions at 75° F. the infections in mosquitoes matured in the relatively short incubation period of 10 days. Under outside conditions in Marysville, the infection developed, but more slowly. Outside in San Francisco, the infections did not develop, but the infections were viable and developed when put in warmer places.

In the past, its association with malaria, its habits of entering houses and biting man, and its prevalence has led to its incrimination as the principal vector in California.

Reeves (1944) gave support to this when he showed by precipitin tests that an average of 3 percent of 473 specimens had fed on man. In one area 7.1 percent had taken human blood meals.

The evidence of its susceptibility reported here supports this incrimination.

*A. punctipennis.* While experiments infecting *A. punctipennis* with plasmodia have been done at other places, it appears that none have been carried out with California or other West Coast specimens. We found the susceptibility to be quite similar to that of *A. m. freeborni* and that sporozoites were developed in 10 days at 75° F. Transmission to man was successful.

In the southeastern states, *A. punctipennis* usually is not considered as being an important vector. It is known, however, to attack man, to be prevalent, and to enter porches and houses.

In California, there are indications on epidemological grounds that it might be a vector of malaria. Lenert (1924), as quoted by Barber, Komp and Hayne (1927), states that *A. punctipennis* is the malaria carrier of the foothills of the Sierra Nevada in California. Herms (1919), suggests *A. punctipennis* as a carrier in Northern California. Simmons and Aitken (1942) quote Herms as saying that this species may play a part in malaria transmission in the Mother Lode Mining region of the western Sierra Nevada foothills of Cali-

fornia.

The experimental evidence obtained by us indicates that it is susceptible to foreign plasmodia.

*A. p. franciscanus*. This species is considered to be of little importance as a vector. Herms (1919) considers *A. pseudopunctipennis* as a weak or no carrier on the coast of California. Barber, et al. (1929) in New Mexico found none of 118 wild-caught *A. psuedopunctipennis* infected. They infected them in the laboratory with *P. vivax* but gave no figures except that *A. maculipennis* gave a larger infection percentage. Barber and Forbich (1933) found none out of 263 (wild-caught) infected in New Mexico. No data on *P. falciparum* or *P. malariae* seem to be available.

Simmons and Aitken (1942) refer to the above as the only experimental work. They reserve the opinion as to whether it was *A. pseudopunctipennis* or *A. p. franciscanus*.

It does not attack man readily. Reeves (1944) out of 178 specimens found only 0.6 percent as having fed on man.

Our experimental evidence indicates that it is susceptible to foreign *vivax* malaria, being similar to *A. m. freeborni* in this respect. However, because of its habits it is not xpected to participate to any extent in the spread of these foreign malarias.

*A. m. occidentalis*. Simmons and Aitken (1942) say there is no experimental evidence of the susceptibility of this species. Neither were the writers able to find any such evidence.

Aitken (1945) believes that it probably plays no part in the transmission of malaria because it occurs in a cool climate.

As to the susceptibility, this report shows it can act as a malaria vector experimentally. As to the climate, recoveries of this species have been made as far south as San Diego on the California coast. Along the southern part of the coast, temperatures are high enough for development of infection in the mosquito. Also summer temperatures in Minnesota and Wisconsin, where this species also occurs, would allow for development of plasmodia in mosquitoes.

It is not supposed to be a house invader. Simmons and Aitken (1942) state that it will feed on man readily in the laboratory.

This was also our experience.

Its prevalence and habits, rather than a lack of susceptibility to plasmodia, appear to be the main reasons why it would not be considered as an efficient vector of foreign malarias. The same conclusion can be applied to its relationship to native malarias.

*Significance of California Anopheles in Spreading Foreign vivax Malaria Relapsing in Returned Carriers*. From the above, *A. p. franciscanus* and *A. m. occidentalis* would not be expected to act as important vectors of imported foreign malaries, even though they are susceptible to the plasmodia.

It is to be expected that *A. m. freeborni* will transmit foreign malarias wherever this mosquito has access to sufficient parasite carriers.

The importance of *A. punctipennis* as a vector is not settled There seems to be reason to expect it to be an efficient vector where it is prevalent enough and malaria carries are available.

It is to be expected that some troops relapsing with foreign malaria eventually will be in areas in California and elsewhere where the above vectors are present. The amount of malaria resulting from such carriers will depend largely upon the control of the mosquito vectors, as no way has yet been found to sterilize the human host of this malaria. With adequate control measures, such outbreaks should be quite limited in number and circumscribed.

*Other Parasitological Considerations.* Infections passed through the mosquitoes to neurosyphilitics were again transmissible through these mosquitoes. There was no indication that the infections were changed by transmission through these insect hosts.

The only *P. falciparum* encountered showed a light infection upon the gut and no gland infection. There is no evidence that *P. falciparum* from foreign areas will fail to infect *A. m. freeborni*. As few cases of *P. falciparum* are expected to be imported by troops, this particular hazard will be not nearly so great as with *P. vivax*. Further observations are being made, however, upon the susceptibility of *A. m. freeborni* to *P. falciparum*.

The outside experiment in San Francisco raised an interesting point concerning the length of time mosquitoes can retain infections. Mosquitoes kept outside did not show development of oocysts for nearly three weeks but upon being put at a warmer temperature, gut infections began to develop. How long can infections in mosquitoes remain viable at cool temperatures in nature? The answer to this question might have a direct relationship to the malaria picture in California.

## *Summary and Conclusions*

1. Mosquito feedings were made on 64 relapsing soldiers and 9 neurosyphilitics who had foreign *Plasmodium vivax* malaria. 89 feedings were made in which 11, 592 mosquitoes (79 per cent) fed out of the 14,640 applied. 63 of the infections were *P. vivax* and one *P. falciparum*. 62 of the *vivax* infections originated from the South Pacific area and one from the Mediterranean area.

2. Of the 64 mosquito lots fed on 62 relapsing *vivax* soldiers from the South Pacific, 48 became infected. In these 48 infected lots, 2,711 *A. maculipennis freeborni* were dissected showing 1,428 (52.67 per cent) infected. This indicates that foreign relapsing *vivax* malaria readily infects *A. m. freeborni*.

3. Attempts were made to transmit by mosquitoes 19 relapsing infections to 23 patients. Seventeen different infections were transmitted to 21 patients. The two failures were Negroes who had had malaria before.

4. With seven different infections, the relapsing soldiers and neurosyphilitics in the induced primary attack showed relatively high infectivity rates to *A. m. freeborni.*

5. A comparison of the infectivity of foreign malarias to four species of Cailfornia mosquitoes (*A. m. freeborni, A. punctipennis, A. maculipennis occidentalis* and *A. pseudopunctipennis franciscanus*) was made. All appeared to have about the same susceptibility. All developed sporozoites. Transmission was tried and successful with *A. m. freeborni, A. punctipennis,* and *A. m. occidentalis.*

6. The length of the sporogonous cycle in the above four species at 75 ° F. was about 10 days. The shortest cycle observed was 8 days in *A. m. freeborni.*

7. Infected mosquitoes were kept at outside temperatures during the last of September and the middle of October. At Marysville, California, the infections developed but much slower than those kept at 75° F. In San Francisco the infections did not develop over a 19-day period; however when put at 75° F. these infections developed showing they were still viable.

Thus one could expect foreign malarias to be spread in climate areas similar to Marysville, particularly in the warmer months, but not in San Francisco, as the observations in the latter were made in what is usually the warmest part of the year there.

8. The gametocyte count in relapsing soldiers averaged 4.5 per 100 w.b.c., ranging from 0 to 32 (0 to 1440 per cmm.).

Two lots of mosquitoes were infected when no gametocytes per 100 w.b.c. were seen. Conversely, a very high gametocyte count of 32 per 100 w.b.c. (1440 per cmm.) did not infect.

Foreign relapsing *vivax* was not always infective to *A. m. freeborni* in direct proportion to the number of gametocytes present. Infections occurred most frequently when there were between 1 and 15 gametocytes per 100 w.b.c (60-900 per cmm.) with the highest infectivity (73.8 per cent of the mosquitoes) within the range of 6-10 gametocytes per 100 w.b.c (360-600 per cmm.).

9. Infection in the mosquitoes usually occurred when exflagellation was demonstrated but also occurred frequently when no exflagellation was seen.

10. In general, gametocytes were produced as long as the relapses occurred. Mosquitoes were infected on patients with the 17th relapse (18th attack). It seems reasonable to expect that some of the patients with foreign *P. vivax* will produce gametocytes and will infect mosquitoes as long as relapses occur.

11. The usual number of oocysts upon the guts of infected *A. m. freeborni* was between 1 and 24. The sporozoites in the glands were usually between 10 and 100. In five cases, all from Guadalcanal, unusually heavy infections were seen showing guts with several hundred oocysts. One gut had approximately 800 oocysts. In these heavy gut infections, the gland infections were corresponding high.

12. In 4 instances, *A. m. freeborni* became infected when applied after malaria therapy with quinine or atabrine had begun. Three of these were *P. vivax* and one *P. falciparum*.

13. The foreign *vivax* malarias were infective to the field-caught as well as to the laboratory-reared *A. m. freeborni*.

14. Foreign *P. vivax* induced in neurosyphilitics produced infections similar to those produced by strains of *vivax* in this country, in particular reference to duration of fevers, fever peaks, parasite densities, and response to thio-bismol. The periodicity of the fevers, in those occurring every other day, was less than 48 hours.

15. On the basis of the evidence presented, it appears justified to conclude that control measures are necessary for the foreign malarias brought to the American west coast by relapsing carriers.

## REFERENCES

Aitken, T. H. G.
1945. Studies on the anopheline complex of Western America. Univ. Calif. Pub. Ent., 7:273-364.

Barber, M. A. and Forbich, L. R.
1933. Malaria in the irrigated regions of New Mexico. Pub. Hlth. Rpts., 48:610-623.

Barber, M. A., Komp, W. H. W., and Hayne, T. B.
1927. The susceptibiltiy to malaria parasites and the relation to the transmission of malaria of the species of *Anopheles* common in the Southern United State Pub. Hlth. Rpts., 42:2485-2502.

Barber, M. A., Komp, W. H. W., and Hayne, T. B.
1929. Malaria and the malaria danger in certain irrigated regions of Southwestern United States. Pub. Hlth. Rpts., 44:1300-1315.

Coatney, G. R., and Young, M. D.
1942. A study of the paroxysms resulting from induced infections of *Plasmodium vivax*. Am. Jour. Hyg., 35:138-141.

Herms, W. B.
1919. Occurence of malaria and anopheline mosquitos in Northern California. Pub. Hlth. Rpts., 34:1579-1587.

James, S. P.
1934. The Shute method of making preparations of exflagellating gametocytes and ookinetes of malaria parasites. Trans. Roy. Soc. Trop. Med and Hyg., 28:104-105.

Lenert, L. G.
1924. Mosquitos and malaria control. Calif. St. Bd. Health Bull. #44. (Quoted by Barber, Komp and Hayne, 1927.)

Reeves, W. C.
1944. Preliminary studies on the feeding habits of Pacific Coast *Anopheles*. Jour. Natl. Mal. Soc., 3:262-266.

Simmons, J. S. S. and Aitken, T. H. G.
1942. The anopheline mosquitoes of the northern half of the Western Hemisphere of the Philippine Islands. Army Med. Bul. #59.

Young, M. D.
1944. Studies on the periodicity of induced *Plasmodium vivax*. Jour. Nat. Mal. Soc., 3:237-240.
Young, M. D., McLendon, S. B., Smarr, R. G.
1943. The selective action of Thio-Bismol on induced malaria. Jour. A. M. A., 122:492-494.
Young, M. D., Stubbs, T. H., Moore, J. A., Ehrman, F. C., Hardman, N. F., Ellis, J. M., Burgess, R. W.
1945. Studies on imported malarias: 1. Ability of domestic mosquitoes to transmit *vivax* malaria of foreign origin. Jour. Nat. Mal. Soc., 4:127-131.

Reprinted from THE JOURNAL OF THE NATIONAL MALARIA SOCIETY
Vol. 6, No. 3, September, 1947

# STUDIES ON IMPORTED MALARIAS: 3. LABORATORY REARING OF WESTERN ANOPHELINES*

NEWTON F. HARDMAN

*Office of Malaria Investigations, National Institute of Health, Columbia, S. C.*

(Received for publication 25 November 1946)

To determine experimentally the ability of western anophelines to become infected with and transmit foreign malaria relapsing in returning troops, a large and continuous production of anophelines was needed (Moore et al 1945). This report details methods used and experience gained in colonizing and producing large numbers of *Anopheles maculipennis freeborni* Aitken. Information gained concerning production of *A. maculipennis occidentalis* (D. & K.), *A. punctipennis* (Say), and *A. pseudopunctipennis franciscanus* (McCracken) is also reported. During the period from January to October, 1944, approximately 89,500 pupae of *A. m. freeborni*, 2,000 pupae of *A. m. occidentalis*, 2,000 pupae of *A. punctipennis*, and 500 pupae of *A. pseudopunctipennis franciscanus* were reared in the insectary located in the Letterman General Hospital, San Francisco, California.

For the purposes of this report, the nomenclature of Aitken (1945) has been followed for these anophelines.

## METHODS

The following methods of rearing larvae and handling pupae and adults were found adequate for a fairly steady production of mosquitoes in the laboratory.

*Insectary.* The insectary was a room of 1,050 cubic feet, the walls and ceiling of which were painted dull white with a fungus resistant paint. Light was obtained from two 48-inch, 40-watt, double tube fluorescent fixtures and 21 x 31-inch north-facing window at ground level. A double curtained trap led to the door. Cages were covered with plastic screen and had cloth sleeves. Larvae were reared in rectangular enameled pans two inches deep and of various outside dimensions. Air temperature was maintained at 28°C. plus or minus 2°C. and the relative humidity between 80 and 95 per cent. Water temperature of rearing pans averaged 27°C.

*Water Used in Raising Larvae.* The tap water available was not found suitable. Although both protozoa and larvae would survive for a few days no larval growth was obtained. Water from a breeding source of *A. m. occidentalis* was found satisfactory for raising the four species of anopheline larvae. Not more than one batch of larvae could be reared in the same water without high mortality although the used water had a higher protozoal content than before use. Distilled water was added to pans to maintain the primary level of 1½ to 2 inches.

* Contribution from the Imported Malaria Studies program of the National Institute of Health and Malaria Control in War Areas, U. S. Public Health Service, Columbia, S. C.

Appreciation is expressed to the staff of the Letterman General Hospital who furnished quarters for the insectary and to the Division of Entomology, University of California, Berkeley, where some preliminary experiments were run.

*Larval Food.* Fresh pond water contained sufficient "food" for newly hatched larvae for two or three days as evidenced by their growth. After this time it was necessary to add supplementary food. Dehydrated, finely ground dog food of various sorts were tried as supplementary food. A mixture of dog food of about 20 per cent protein and 10 per cent dry brewer's yeast was found satisfactory.

After grinding and mixing, the food was sifted through suitable material to exclude the larger particles. Food was dusted on the surface of water when the surface became apparently free of bacterial "scum" or of food particles. Differential hatchability of each batch of eggs and mortality of larvae from one pan to another necessitated treating each pan of larvae as a separate problem as to feeding frequency and amount fed.

*Technique of Handling Eggs, Larvae, and Pupae.* A heavy, gelatinous "scum" formed on the pond water surface within a few hours after the water was poured into breeding pans. Because this "scum" trapped and killed newly hatched larvae it was found necessary to incubate eggs 2 days in a small bowl or half-pint carton and then rinse the eggs into pans of fresh pond water. In this way the small larvae consumed the "scum" as it formed without becoming trapped. It was found that approximately 200 larvae per square foot of water surface could be reared through the second instar. However, after the second instar 200 larvae per square foot would consume food so rapidly that overnight they exhausted all food particles that could be loaded on the water surface and then consumed the setae on one another. When conditions permitted larvae were given more surface area.

A large flagellated rod bacterium was frequently found at the water surface in larval pans associated with very high mortality of larvae. Dissections of larvae obviously affected by something in these "cultures" showed this bacterium packed in the alimentary canal and alive as evidenced by their motility when expressed and examined. Larvae which had recently died were found to have large numbers of this bacterium in their body tissues. To reduce this hazard, pipettes used for removing pupae and handling larvae were rinsed with alcohol between usage in order to prevent contamination from one pan to another.

Pupae were removed once daily to half-pint cardboard cartons and rinsed with clear water to free them of larval "culture." If pupae were not rinsed a heavy gelatinous "scum" formed in which large numbers of adults became trapped during emergence resulting in some instances in 100 per cent mortality. After rinsing two or three large larvae were put in each carton to consume any "scum" that formed.

## RESULTS

With the above techniques and under the pressure of trying to obtain as many pupae as possible with the space limitations, it was noted that in the first and subsequent generations the body size of larvae, pupae, and adults was obviously smaller than that of field-collected specimens. This was true with all species reared from eggs as well as with *A. m. occidentalis* reared from field-collected first, second, and third instar larvae. A few experiments in which 10 *A. m. freeborni* larvae were reared per square foot instead of 100 to 200 showed that given sufficient water

surface area and reduced competition for food, larvae, pupae, and adults were produced that were approximately the same size as those collected in the field.

During a period of 92 consecutive days 39,750 *A. m. freeborni* pupae were obtained from approximately 74 square feet of water surface. The time interval from egg laying until the formation of the last pupae was about 21 days at 27°C. (Table 1).

A check of samples of *A. m. freeborni* pupae totalling 21,389 revealed that 4 per cent failed to emerge successfully.

*A. m. freeborni.* Progeny of field-collected females from Riverside, Merced, and Auburn, California, were reared and mated successfully in 10, 12, and 20-inch cubed cages during a period from January to March. No gross differences were found

TABLE 1

*Comparison of Different Generations of* A. m. freeborni *at 27°C. as to Rate of Development and Variations*

| GENERATIONS REMOVED FROM FIELD-CAUGHT FEMALES | NO. LOTS* EXAMINED EACH GENERATION | TOTAL NO. PUPAE | DAY OF 1ST PUPAE | | DAY ½ WERE PUPAE | | DAY ¾ WERE PUPAE | | DAY ALL WERE PUPAE | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Av. | Range | Av. | Range | Av. | Range | Av. | Range |
| Eggs from Field Females... | 25 | 5,114 | 13.1 | 11–19 | 16.7 | 12–24 | 18.1 | 14–26 | 20.6 | 15–28 |
| 1st..... | 30 | 6,127 | 14.8 | 11–21 | 18.8 | 13–24 | 20.3 | 15-16 | 22.4 | 15–28 |
| 2nd..... | 31 | 3,679 | 13.2 | 10–18 | 15.3 | 10–22 | 16.2 | 11–24 | 18.9 | 12–30 |
| 3rd..... | 31 | 5,586 | 13.2 | 11–17 | 15.6 | 11–22 | 16.7 | 11–22 | 18.6 | 13–26 |
| 4th..... | 26 | 3,445 | 13.4 | 11–17 | 15.2 | 11–21 | 16.3 | 13–19 | 17.9 | 14–25 |
| 5th..... | 16 | 1,914 | 15.4 | 11–19 | 20.3 | 13–27 | 22.2 | 15–28 | 25.6 | 15–28 |
| 6th..... | 28 | 3,024 | 14.5 | 12–19 | 18.2 | 15–22 | 19.7 | 16–24 | 22.3 | 18–26 |
| Total of 6 Generations... | 162 | 23,775 | | 10–21 | | 10–27 | | 11–28 | | 12–30 |
| Average of 6 Generations..... | | | 14.1 | | 17.2 | | 18.6 | | 20.95 | |

* In this table and subsequently, a lot is defined as a quantity of eggs put in a single pan. The quantity was varied with the size of the pan.

between the groups from these different areas. Because of the relative abundance of this species at Marysville, California, females were collected there and were used to obtain a permanent colony. This colony was continued from March to October without the necessity of adding new stock from the field. During this time seven generations were completed. No attempt was made to obtain as many generations as possible in this period.

To obtain mating and eggs, 500 to 1,000 pupae were introduced into plastic-screened cages 20 inches cubed. Adults that emerged (from 1 to 3 days after being collected) were given freshly cut surface of apples each evening until spermathecal examination of a sample of females showed over one-half inseminated. Without apple or some other source of sugars no insemination occurred. Usually 10 to

15 days contact with males was necessary before one half of the females were inseminated. In a few batches after 10 days of contact with males, all females dissected were found inseminated. No relationship could be found between blood feeding of females and insemination or between insemination and ovarian development. After females were inseminated they were fed on man and/or rabbit. By using four mating cages and staggering the introduction of pupae a fairly steady production of eggs resulted.

A pad of cloth large enough to cover the top of the mating cage and saturated with 5 per cent glucose solution was found superior to apple feeding in the resultant number of females producing eggs. It was found necessary to wash the pad thoroughly each day to prevent the development of yeast.

The production of eggs was increased by placing 10 to 15 inseminated females over water in small carton cages and by giving them an opportunity to feed on man every day. By this method, about 20 per cent of the total pupal production was necessary for the number of eggs needed.

In a single experiment, 20 non-blood-fed females selected at random from mating cages were placed in half-pint carton cages covered on both ends with bobbinet. A pad of cotton was saturated with either water or 5 per cent glucose in water and placed on top of the bobbinet. The cages were put in a refrigerator held at 4°C. and the pads renewed or resaturated when necessary. It was found that when water only was available one half were dead in 64 days (average length of life 40 days) whereas if 5 per cent glucose solution was available one half were not dead until 124 days (average length of life 64 days). Apparently, the females were able to move and imbibe the glucose solution and water at this temperature (?) as their crops were frequently found distended with a clear liquid. Perhaps they moved and fed when the temperature was elevated temporarily when the door was opened. Upon the basis of this experiment, continuous egg production was obtained by routinely refrigerated females.

Refrigeration at 4°C. of freshly laid eggs was not found practical beyond 10 days as after this time the number hatching fell rapidly to zero.

The difference in hatchability of eggs from field-collected females compared with the eggs from successive generations of laboratory-reared females (Table 2) is difficult to understand considering the finding that extremely few females laid eggs when not inseminated even though replete with them.

A comparison of survival to the pupal instar (Table 3) and rate (Table 1) of development of laboratory-reared *A. m. freeborni* showed no definite relationship with the generation. True relationships may be masked by the relatively low, 58 per cent, (Table 2) survival of larvae.

Since the gross size of laboratory-reared adults and the hatchability of their eggs were different from the field-collected females, it was considered essential to compare field-collected females and laboratory-reared females in other respects.

Table 4 summarizes the results of two experiments carried on at 28°C. comparing laboratory-reared females randomly selected with randomly collected females (near Marysville, California). The first collection in April of field females is compared in Table 4 with second generation females from the laboratory; the second collection in

September divided into two groups is compared with the fourth generation from the laboratory. All females were confined separately in carton cages over water and given an opportunity to feed on man at least every other day. Some of the field-

TABLE 2

*Hatchability of Eggs of Various Generations of* A. m. freeborni *at 27°C.*

| GENERATIONS REMOVED FROM FIELD FEMALES | LOTS CHECKED | EGGS COUNTED | LARVAE HATCHED | PER CENT HATCHED | SURVIVAL OF THOSE HATCHING | | |
|---|---|---|---|---|---|---|---|
| | | | | | Sample Size | Number Pupae | Per Cent Pupated |
| By field females | 1+ | 2,500* | 2,000* | 80† | | | |
| 2nd | 16 | 6,695 | 3,278 | 49.0 | 3,278 | 1,772 | 53.9 |
| 3rd | 109 | 46,078 | 24,375 | 52.9 | 21,494 | 13,547 | 63.0 |
| 4th | 15 | 5,603 | 3,492 | 62.3 | 3,015 | 1,505 | 49.9 |
| 5th | 20 | 8,615 | 4,632 | 53.8 | 4,632 | 1,915 | 41.3 |
| Total of 2, 3, 4, and 5th generations | 160 | 66,991 | 35,777 | 53.4 | 32,419 | 18,739 | 57.8 |

+—One night's laying of many randomly collected field females.

* Estimated.

† Approximately.

Survival data were selected to exclude information from those pans infected with the deleterious bacterium, described above.

TABLE 3

*Comparison of Survival of* A. m. freeborni *of Different Generations from Egg to Pupae at 27°C.*

| GENERATIONS REMOVED FROM FIELD FEMALES | NUMBER LOTS | NUMBER EGGS | NUMBER PUPAE | PER CENT PUPATING |
|---|---|---|---|---|
| By field females | 25 | 11,843 | 5,114 | 43.2 |
| 1st | 30 | 12,452 | 6,127 | 49.2 |
| 2nd | 31 | 9,989 | 3,679 | 36.8 |
| 3rd | 31 | 10,780 | 5,586 | 51.8 |
| 4th | 26 | 10,356 | 3,445 | 33.2 |
| 5th | 16 | 6,131 | 1,914 | 31.2 |
| 6th | 28 | 7,915 | 3,024 | 38.2 |
| Total for 6 Generations | 162 | 57,623 | 23,775 | 41.3 |

Data are from females collected separately and colonized separately from those reported in Table 2.

Data were selected to exclude those lots where the water was found infected with the deleterious bacterium described above.

collected females must have had at least one blood meal previously whereas laboratory-reared females had no blood before being placed in the cages. Although the samples studied were admittedly small they were nevertheless chosen at random.

It is interesting that although the previous history of the field females as to age and nutrition was not known, they lived about as long after being transported in a cage 120 miles as reared females and laid on the average considerably more eggs per laying and per life than did the laboratory-reared females.

The maximum number of eggs deposited by one field-collected female *A. m. freeborni* was 1,527 and the average for 34 females was 751 eggs in their lifetime, or 176 per laying. Two females at one oviposition laid 432 and 408 respectively (Table 4). These results may be compared with the findings of Herms and Freeborn

TABLE 4

*Comparison of Field-Collected with 2nd and 4th Generation Laboratory-Reared* A. m. freeborni *at 28°C. as to Longevity, Egg Production, and Number of Blood Engorgements*

| MATERIAL AND CONDITIONS | NUMBER FEMALES STUDIED | DAY ½ DEAD | MAX. LENGTH LIFE DAYS | AV. LENGTH LIFE DAYS | AV. NO. EGGS PER FEMALE PER LAYING | AV. NO. EGGS PER FEMALE | GREATEST NO. EGGS | | ENGORGEMENTS | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | By one Female | At one Time | Av. Per Female | Greatest No. by one Female |
| Field, April | | App. | | | | | | | | |
| Water & Blood | 18 | 27 | 41 | 25.0 | 194 | 798 | 1,527 | 432 | 7.5 | 20 |
| Field, Sept. | | | | | | | | | | |
| Water & Blood | 8 | 27 | 48 | 25.0 | 192 | 768 | 1,388 | 409 | 5.5 | 10 |
| Field, Sept. | | | | | | | | | | |
| 5% Glucose & Blood | 8 | 21 | 40 | 29.8 | 148 | 686 | 1,129 | 308 | 5.9 | 14 |
| Averages..... | 34 | 25 | 44.2 | 26.6 | 176 | 751 | | | 6.3 | |
| 2nd Generation | | | | | | | | | | |
| Water & Blood | | | | | | | | | | |
| Laid eggs | 8 | 24 | 36 | 28.0 | 101 | 339 | 734 | 183 | 7.0 | 12 |
| Laid no eggs | 11 | 18 | 31 | 19.0 | | | | | 4.8 | 9 |
| 4th Generation | | | | | | | | | | |
| Water & Blood | | | | | | | | | | |
| Laid eggs | 11 | 29 | 36 | 27.7 | 130 | 440 | 707 | 279 | 5.7 | 8 |
| Laid no eggs | 8 | 23 | 29 | 23.0 | | | | | 4.8 | 6 |
| Averages of Those Laying Eggs..... | 19 | 26.5 | 36 | 27.8 | 115.5 | 389.5 | | | 6.3 | |
| Averages of Those Not Laying Eggs..... | 19 | 20.5 | 30 | 21.0 | | | | | 4.8 | |

(1920) who report a maximum of 315 eggs laid by a field-collected female and for 30 females—an average of 209 eggs. In subsequent work Herms and Frost (1932) report a maximum of 288 and an average of 195. Aitken (1945) reports a maximum of 268 for one laying and an average of 106 from females collected at Sunol and in the Sacramento and San Joaquin Valleys. He also found a maximum of 149 and an average of 95 for females collected at Point Reyes station, Marin County, Valley Ford, Sonoma County, and Castroville, Monterey County.

*A. m. occidentalis.* Two attempts at colonization of the species failed in 1943 and 1944 with progeny of field-collected females reared in the laboratory. Females were

collected at Hamilton Field, Palo Alto and an area 13 miles south of San Francisco in irrigation reservoirs occurring near the ocean. Cages tried were 20 inches cubed, 54 x 28 x 28 inches, and 5 x 5 x 12 feet. The first two cages were tried in the insectary and the last one at the University of California Laboratory of Insect Physiology. Although both sexes fed readily on fruits, honey and sugar solutions (as does *A. m. freeborni*) and the females fed readily on man, only one batch of eggs was laid, none of which hatched. Dissection of a few of these eggs showed no embryos.

On one occasion, 12 females were collected in the field but only 6 laid eggs when confined singly in carton cages. The greatest number of eggs laid by one female was 894 during its life in the insectary when fed blood at least every other day. The greatest number of eggs laid at one time by one female was 333. The average per laying was 202 and the average per female during life under caged conditions was 337.

Examination of a quantity of freshly emerged progeny of positively identified field-collected *A. m. occidentalis* revealed no intergradations of the pale wing tache with the unicolorous condition found in *A. m. freeborni*.

*A. punctipennis*. Three attempts to colonize *A. punctipennis* from Auburn and Hamilton Field, California, failed. No particular difficulty was experienced in rearing progeny of field-collected females, but attempts to satisfy the conditions for mating failed. Adults fed readily on fruit and 5 per cent glucose solution and females fed on blood repeatedly but laid no eggs although their ovaries were mature.

*A. pseudopunctipennis franciscanus*. Two of three attempts with this species to obtain mating in cages were successful. Females for oviposition were collected in Marysville and Palo Alto, California. The size of cage seemed to have some influence as the smallest cage in which mating was successful was 14 x 14 x 36 inches. It was found necessary to blood-feed females at least once before insemination occurred. Furthermore, no females were found inseminated until all traces of the previous blood meal were gone from the mid gut. This is in contrast to *A. m. freeborni* where females mated without reference to blood feeding. A small colony was carried through two generations without any special difficulties except in inducing females to feed on man or rabbit. Many females would die of starvation before they would feed on man or the rabbit. However, they were highly attracted to a cow and would engorge rapidly when applied. This tends to substantiate Reeves' (1944) tentative conclusion that "cow appeared to be its preferred host." The colony was stopped as no further use was found for specimens.

A number of eggs would frequently sink to the bottom of the water in which they were laid. These sunken eggs would hatch but required from 2 to 4 days longer than the ones that remained at the surface. Both *boydi* and *franciscanus* types of eggs figured by Aitken (1945) were produced from a single first generation laboratory-reared female. Some of these eggs exhibited a form taken to be intermediate between the two types.

## SUMMARY AND CONCLUSIONS

1. A colony of *Anopheles maculipennis freeborni* was maintained for a 7-month period during which time approximately 89,500 pupae were obtained and 7 generations were passed through.

2. *A. m. freeborni* mated readily in cages as small as 10 inches cubed if utilizable carbohydrates were available.

3. Water from a natural breeding site of *A. m. occidentalis* was found suitable for rearing larvae of all species whereas the tap water available was not suitable.

4. A supplementary food composed of 10 per cent dry brewer's yeast and 90 per cent finely ground dehydrated dog food with a total protein content of about 20 per cent was found adequate for feeding larvae.

5. An average of approximately 122 larvae of *A. m. freeborni* were reared per square foot of water surface 1½ to 2 inches deep. A batch of larvae required on an average of about 21 days from oviposition to the pupation of the last larvae. The range was from 12 to 30 days at an average water temperature of 27°C.

6. *A. m. freeborni* survival from first instar to pupae under insectary conditions was approximately 58 per cent.

7. Pupal mortality was reduced from a high percentage to approximately 4 per cent when the pupae were washed free of larval "culture" and large larvae were added to reduce bacterial "scum."

8. No consistent difference was found in the rate of development between laboratory-reared progeny of field-collected *A. m. freeborni* and successive generations.

9. Laboratory-reared *A. m. freeborni* were not equivalent to field-collected adults as to gross body size, when 100 to 200 were reared per square foot of surface area. At 10 per square foot, the size was about the same as field-collected specimens. The average number of eggs laid per lifetime per female and the average hatchability of eggs laid were less in the laboratory-reared than in the field-collected females.

10. Two successive generations of *A. pseudopunctipennis franciscanus* were obtained without difficulty when the cage was 14 x 14 x 36 inches or larger and when females were fed at least once upon blood.

11. No evidence was obtained of *A. m. occidentalis* or *A. punctipennis* mating under conditions suitable for *A. m. freeborni*.

ACKNOWLEDGMENT

The author is indebted to Dr. Martin D. Young for his valuable aid and criticism in the preparation of this paper.

## REFERENCES

Aitken, T. H. G. 1945. Studies on the Anopheline Complex of Western America. Univ. of Calif. Publications in Entomology. Vol. 7, No. 11, 273–364 (39 figs.).

Herms, W. B. and Freeborn, S. B. 1920. The Egg-Laying Habits of California Anophelines. Jour. Parasitology. **7:** 69–79.

Herms, W. B. and Frost, F. M. 1932. A Comparative Study of California Anophelines. Jour. Parasitology. **18:** 240–244.

Moore, J. A., Young, M. D., Hardman, N. F. and Stubbs, T. H. 1945. Studies on Imported Malarias: 2. Ability of California Anophelines to Transmit Malaria of Foreign Origin and Other Considerations. Jour. Nat. Mal. Soc. Vol. IV, No. 4, 307–329.

Reeves, W. C. 1944. Preliminary Studies on the Feeding Habits of Pacific Coast Anophelines. Jour. Nat. Mal. Soc. Vol. IV, No. 4, 261–266.

Reprinted from THE AMERICAN JOURNAL OF HYGIENE, Vol. 43, No. 3,
326–341, May, 1946
Printed in U. S. A.

# STUDIES ON IMPORTED MALARIAS. 4. THE INFECTIVITY OF MALARIAS OF FOREIGN ORIGIN TO ANOPHELINES OF THE SOUTHERN UNITED STATES

# STUDIES ON IMPORTED MALARIAS. 4. THE INFECTIVITY OF MALARIAS OF FOREIGN ORIGIN TO ANOPHELINES OF THE SOUTHERN UNITED STATES [1]

By

MARTIN D. YOUNG, TRAWICK H. STUBBS, JOHN M. ELLIS, ROBERT W. BURGESS, AND DON E. EYLES [2]

(Received for publication January 12th, 1946)

## Introduction

Observations on the ability of domestic mosquitoes to transmit *Plasmodium vivax* of foreign origin were presented in a preliminary report (Young et al, 1945) which covered the first year's work of the *Imported Malaria Studies* program. A second report (Moore et al, 1945) described in detail the ability of Western anophelines, particularly *Anopheles maculipennis freeborni,* to transmit these infections.

The present report presents detailed observations on the ability of Southern anophelines to become infected with malarias of foreign origin showing clinical relapses in returned troops. The observations covered the 18-month period, ended June 2nd, 1945. Laboratories were maintained at Harmon General Hospital, Longview, Tex., Moore General Hospital, Swannanoa, N. C., and the South Carolina State Hospital, Columbia, S. C.

## Methods

The procedures followed have been described in earlier reports (Young, Stubbs et al, 1945; Moore et al, 1945; and Burgess and Young, 1944). In general, they consisted in feeding mosquitoes (preferably 100 or more) upon patients with relapsing malaria; dissecting the mosquitoes at intervals to determine the rates of infection; and demonstrating transmission by feeding selected lots upon neurosyphilitic patients requiring malaria therapy.

An infection in the mosquito is defined as the presence of oocysts or sporozoites, or both.

At the Texas and North Carolina laboratories, feedings were usually made on patients in whom gametocytes had been demonstrated. At the Columbia, S. C., laboratory, mosquitoes were applied to relapsing patients without previously demonstrating the presence of gametocytes.

After the infective feeding, the mosquitoes were placed in an insectary with the temperature controlled between 74 and 80 F and a high relative humidity.

Insectaries were maintained at the 3 laboratories to furnish a continuous supply of our Q-1 strain of *A. quadrimaculatus.* The other species were mainly field-caught adults or the first few generations therefrom.

More than one million mosquitoes were handled, as follows: 982,613 *A. quadrimaculatus;* 3,170 *A. punctipennis;* 1,075 *A. pseudopunctipennis pseudopuncti-*

[1] Contribution from the Imported Malarial Studies program of the Office of Malaria Investigations, National Institute of Health and the Office of Malaria Control in War Areas, United States Public Health Service.

[2] The following hospitals made relapsing cases available: the Harmon General, Moore General, Oliver General, Stark General, Fort Jackson (S. C.) Regional, and Charleston (S. C.) Naval. To these and especially to Harmon General, Moore General, and the South Carolina State Hospital, which also furnished the laboratory quarters, we express appreciation. We are also indebted to the Office of The Surgeon General, United States Army, whose active interest made the program possible.

TABLE 1

*Foreign origin of relapsing* P. vivax *infectious in returned troops and infectivity to* A. quadrimaculatus

| Probable origin of infection | Patients upon whom mosquitoes were fed* | | Mosquitoes fed on patients | | | |
|---|---|---|---|---|---|---|
| | | | Lots | | Mosquitoes | |
| | Number | Number infective to mosquitoes | Fed | Infected | Dissected | Per cent infected |
| Guadalcanal | 68 | 41 | 71 | 43 | 2,724 | 28.9 |
| New Guinea | 43 | 22 | 48 | 25 | 1,823 | 34.7 |
| Other South Pacific† | 6 | 2 | 6 | 2 | 210 | 3.8 |
| Total South Pacific | 117 | 65 | 125 | 70 | 4,757 | 30.1 |
| Mediterranean | 40 | 30 | 40 | 30 | 1,256 | 37.2 |
| Caribbean | 6 | 4 | 6 | 4 | 147 | 13.6 |
| Burma‡ | 1 | 0 | 1 | 0 | 40 | 0.0 |
| Liberia | 1 | 1 | 1 | 1 | 47 | 21.3 |
| Totals (all areas) | 165 | 100 | 173 | 105 | 6,247 | 30.8 |

* In some cases, multiple feedings were made on one patient so that a total of 173 lots of mosquitoes were fed on 165 patients.

† Includes New Georgia, New Hebrides, Tulagi, Munda, Biak.

‡ A second case from Burma has produced infections in mosquitoes.

*pennis;* 3,731 *A. walkeri,* and 13,014 *A. albimanus.*

Three hundred and ten lots of *A. quadrimaculatus* were fed upon patients with *Plasmodium vivax* infection of foreign origin as follows: relapsing soldiers, 173; neurosyphilitics with induced malaria, 137. In these lots, there were 36,788 mosquitoes applied, of which 30,794 (83.7 per cent) fed.

Two cases of *Plasmodium falciparum* infection of foreign origin were also studied, involving 261 *A. quadrimaculatus* applied, of which 224 (85.8 per cent) fed.

## OBSERVATIONS

### *Origin of the relapsing malarias and infectivity to* A. quadrimaculatus

The probable origins of the relapsing infections are shown in table 1. Most of them appeared to originate in the South Pacific, from Guadalcanal and New Guinea.

It is seen from table 1 that *P. vivax* infections from widely scattered areas of the world were infective to *A. quadrimaculatus.* The two areas from which most of the infections originated, i.e., the South Pacific and the Mediterranean, seemed quite similar in their infectivity.

The data on the two cases of *P. falciparum* infection are shown in table 2.

TABLE 2

*Infectivity of foreign relapsing* P. falciparum *infection to* A. quadrimaculatus

| Infection number | Gam./100 w.b.c. | Mosquitoes | | | |
|---|---|---|---|---|---|
| | | Applied | Fed | Dissected | Infected |
| 6-Af* | 0.2 | 141 | 117 | 10 | 2 |
| 1513-G | 68.0 | 120 | 107 | 28 | 0 |

* Af = Northern Africa; G = Guadalcanal.

It is believed that a slightly higher proportion of mosquitoes became infected than appears from tables 1 and 2 for *P. vivax* and *P. falciparum* infections. During the first part of the work, when dissections were made, the mosquitoes which had not taken a blood meal and had survived were dissected along with those which had taken blood meals. During the latter part of the work, however, all mosquitoes which did not feed were killed. Although the difference resulting from this change of procedure is undoubtedly slight, the infection rates as shown represent the minimum infectivity.

The fact that 61 per cent of the lots of mosquitoes fed upon the relapsing *P. vivax* infections showed some infected mosquitoes and that 31 per cent of all mosquitoes which fed became infected, indicates that these foreign *P. vivax* malarias readily infect *A. quadrimaculatus*. The data on the *P. falciparum* malaria are too limited to warrant any conclusions except that some infection did occur.

For final proof of transmission, selected lots of infected mosquitoes were applied to neurosyphilitic patients requiring malaria therapy. Transmission was successful in most of the attempts (Young, Ellis, and Stubbs, 1946).

### *Comparative susceptibility of two strains of* A. quadrimaculatus

The strain of *A. quadrimaculatus* used routinely has been maintained in the Columbia, S. C., laboratory for over 3 years, and has been designated by us as "Q-1." It probably represents a hybrid of stock colonies secured by the Malaria Investigations office in Memphis, Tenn., from the Tennessee Valley Authority at Wilson Dam, Ala., and the Rockefeller Institute of Tallahassee, Fla. The latter colony has been maintained for over 13 years.

To compare it with a colony more recently secured from nature, a colony was started in Longview, Tex., in the late fall of 1944, from adult mosquitoes obtained adjacent to the Harmon General Hospital. This was designated as the "Q-3" strain.

Lots of both "Q-1" and "Q-3" strains were applied simultaneously to patients with foreign malaria. The data on 19 such feedings are shown in table 3.

TABLE 3

*Comparative susceptibility of two strains of* A. quadrimaculatus *to* P. vivax. *infections of foreign origin, both strains simultaneously fed*

| | *A. quadrimaculatus* strains | |
|---|---|---|
| | *Q*−1 | *Q*−3 |
| Number of feedings | 19 | 19 |
| Different infections | 6 | 6 |
| Mosquitoes dissected | 429 | 338 |
| Mosquitoes infected | 355 | 283 |
| Per cent infected | 82.7 | 83.7 |
| Average oocyst intensity* | 2.6 | 2.7 |

* In designating intensity, oocysts were grouped as follows: + = 1 − 9 oocysts; ++ = 10 − 24; +++ = 25 − 99; ++++ = 100 and over. These intensity groups were averaged and expressed as numbers, i.e., the average of one + and one ++ is expressed as 1.5.

The data presented in table 3 indicate that the percentage of infections and the intensity of infections were similar in both strains of *A. quadrimaculatus* tested against *P. vivax* infections of foreign origin. It is of interest that the two colonies tested originated from widely separated sources.

Boyd (1939) has shown that long colonization of his strain of *A. quadrimaculatus* did not alter the strain's susceptibility to American malarias. These

findings support those of Boyd which indicate that a long established colony might continue to have a susceptibility similar to that of mosquitoes recently colonized. By analogy, they also suggest that the susceptibility of the insectary mosquitoes is representative of those in nature.

*The infectivity to* A. quadrimaculatus *of 15* P. vivax *malarias of foreign origin relapsing in soldiers compared to the infectivity of the same infections induced in neurosyphilitic patients*

Further tests of the infectivity of these foreign malarias were made by feeding mosquitoes upon neurosyphilitic patients to whom the infections had been transmitted. In some patients, the infection had been induced by inoculation with infected blood; in the remainder the infections had been transmitted by the bites of infected mosquitoes. Some of the latter infections had been through several man-mosquito serial transfers.

The mosquitoes were fed upon the neurosyphilitic patients during the primary attack of the induced infection. In the soldiers, the feedings occurred during either the relapses or delayed primary attacks.

The data on these feedings are given in table 4.

The gametocyte counts in the neuro-

TABLE 4

*Infectivity to* A. quadrimaculatus *of 15* P. vivax *malarias of foreign origin relapsing in soldiers compared to the same infections induced in neurosyphilitic patients*

| Infection number | Neurosyphilitic patients | | | | | Relapsing soldiers | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. trials | Average no. gametocytes* | Mosquitoes | | | No. trials | Average no. gametocytes* | Mosquitoes | | |
| | | | Infected | Dissected | Per cent infected | | | Infected | Dissected | Per cent infected |
| 1005-G† | 5 | 4.2 | 76 | 131 | 58.0 | 2 | 4.0 | 36 | 79 | 45.6 |
| 1017-NG | 2 | 4.5 | 17 | 48 | 35.4 | 1 | 11.0 | 29 | 74 | 39.1 |
| 1019-G | 2 | 4.5 | 45 | 69 | 65.2 | 1 | 2.0 | 34 | 70 | 48.6 |
| 1023-Si | 1 | 5.0 | 6 | 20 | 30.0 | 1 | 3.0 | 30 | 62 | 48.4 |
| 1027-NG | 56‡ | 5.2 | 975 | 1,916 | 50.8 | 3 | 5.0 | 93 | 196 | 47.4 |
| 1030-NG | 1 | 1.0 | 36 | 54 | 66.6 | 2 | 4.0 | 40 | 64 | 62.5 |
| 1031-Si | 23 | 5.5 | 397 | 621 | 63.9 | 1 | 3.0 | 53 | 61 | 86.9 |
| 1032-NG | 11 | 5.8 | 161 | 242 | 66.5 | 1 | 2.0 | 30 | 36 | 83.3 |
| 1033-NG | 1 | 4.0 | 0 | 26 | 0.0 | 1 | 10.0 | 34 | 38 | 89.5 |
| 1035-Af | 1 | 2.0 | 5 | 20 | 25.0 | 1 | 4.0 | 16 | 63 | 25.4 |
| 1037-Si | 2 | 7.5 | 37 | 45 | 82.2 | 1 | 3.0 | 10 | 17 | 58.8 |
| 1040-Si | 2 | 2.0 | 45 | 70 | 64.2 | 1 | 8.0 | 26 | 33 | 78.7 |
| 20-Si | 1 | 18.0 | 2 | 40 | 5.0 | 1 | 1.0 | 16 | 31 | 51.6 |
| 21-G | 3 | 5.0 | 13 | 72 | 18.0 | 1 | 2.1 | 23 | 25 | 92.0 |
| 22-Tu | 1 | 34.3 | 10 | 15 | 67.0 | 1 | 1.7 | 17 | 24 | 70.8 |
| Totals and averages | | | | | | | | | | |
| 15 | 112 | 5.6 | 1,825 | 3,389 | 53.9 | 19 | 4.3 | 487 | 873 | 55.8 |

* Average number of gametocytes per 100 white blood cells.

† G = Guadalcanal; NG = New Guinea; Si = Sicily; Af = Northern Africa; Tu = Tunisia.

‡ 26 of these feedings were upon persons with infections which had been induced by blood transfer.

syphilitic patients averaged 30 per cent higher than in the relapsing soldiers. However, the rate of infection in the mosquitoes was virtually the same for both groups, being slightly higher in the lots fed upon the relapsing soldiers.

These results indicate that the *P. vivax* malarias, originating in either the Pacific or Mediterranean areas, were still infective to our mosquitoes after passage by blood transfers and by *A. quadrimaculatus*, and at about the same rate as with the relapsing soldiers. The insect vector was different from the ones in the areas where the infections originated. However, the parasite apparently readily adapted itself to a new insect vector.

### *Relationship of number of* P. vivax *gametocytes to infection in* A. quadrimaculatus

In all cases, gametocytes were counted and expressed as the number per 100 white blood cells. In 50 instances (Texas laboratory), white blood cell counts were made at the time of feeding of the mosquitoes and in these the number of gametocytes per cu. mm. of blood was calculated also. A graph was plotted to compare the above mentioned two methods of counting and it was found that the two curves correlated closely. The white blood cell counts in the 50 cases averaged 6,400 per cu. mm.

The relationship of the mosquitoes infected to the number of gametocytes present is shown in table 5.

From these data it is seen that a sharp rise in the rate of infection occurred when one or more gametocytes per 100 white blood cells (64 per cu. mm.) were present.

The highest infection rates occurred when most gametocytes (16 to 25 per 100 white blood cells) were present. However, as there were only a few feedings involved with the higher gametocyte counts, this relationship may be more apparent than real.

TABLE 5

*Relationship of number of gametocytes per 100 white blood cells to* A. quadrimaculatus *infected in 173 feedings upon relapsing* P. vivax. *infection of foreign origin*

| Gametocytes per 100 w.b.c. | Mosquito lots | | Mosquitoes | | |
|---|---|---|---|---|---|
| | Fed | Infected | Dissected | Infected | Percentage infected |
| Less than 1 | 55 | 12 | 1,747 | 134 | 7.7 |
| 1–5 | 86 | 64 | 3,378 | 1,268 | 37.5 |
| 6–10 | 20 | 18 | 672 | 306 | 45.5 |
| 11–15 | 6 | 5 | 239 | 90 | 37.7 |
| 16–20 | 4 | 4 | 122 | 67 | 55.9 |
| 21–25 | 2 | 2 | 89 | 61 | 68.5 |
| Totals and averages 3.4 | 173 | 105 | 6,247 | 1,926 | 30.8 |

Of the 173 cases, 86 (about one-half) showed between 1 and 5 gametocytes per 100 white blood cells. The total average was 3.4 gametocytes. Previously, 65 cases had been reported from California (Moore et al, 1945), in which the average gametocyte count was 4.5 per 100 white blood cells and, as with the present group, a large proportion (41 of 65) of the cases showed from 1 to 5 gametocytes per 100 white blood cells.

In 5 instances, when there was less than 1 gametocyte per 100 white blood cells, the infection rate in *A. quadrimaculatus* was 65 per cent or more, which is a relatively high rate.

Conversely, in several cases a high gametocyte count resulted in low infection rates, viz., two cases showing 21 and 13 gametocytes per 100 white blood cells produced infection rates of 6 and 7 per cent, respectively, in the mosquitoes.

Therefore, it appears that the following conclusions can be drawn. In relapsing *P. vivax* infection of foreign

origin, in general, an increase in the number of gametocytes resulted in increase of infection rates in *A. quadrimaculatus,* with the sharpest increase occurring at one gametocyte per 100 white blood cells. There were exceptions to the above in both directions.

### *Relationship of the number of relapses to duration of the infection, to the number of gametocytes produced, and to the infectivity to mosquitoes*

When mosquitoes were fed upon a patient, an effort was made to determine the time since the primary attack and also how many attacks the patient had experienced subsequently. The first attack was designated as the primary attack regardless of its relationship to suppressive treatment. In many instances, the primary attack had been delayed considerably by the taking of suppressive drugs. The next attack was designated as the first relapse and subsequent relapses were numbered in order of their occurrence.

Blood smears taken at the time of feeding mosquitoes, or shortly before, revealed the gametocyte density.

These data, together with the rate of infection in the mosquito, are shown in table 6 and in figure 1.

A breakdown of the data presented in table 6 for the two principal areas, the Pacific and Mediterranean, gives the following average figures, respectively: gametocytes per 100 white blood cells, 3.3 and 3.9; relapses, 8.5 and 7.1; duration of infection in months, 15.7 and 13.4; per cent of mosquitoes infected, 30.1 and 37.2.

From the viewpoint of transmission, it is important to know how long relapses will occur in malarious patients. As seen from table 6, in 146 cases the average duration of the disease had been 15 months, and during this time an average of 8 paroxysms had been experienced. Furthermore, there seemed to be a wide variation in the frequency of relapses in individuals. Whereas one patient experienced 26 relapses in 22 months, others had only 3 or 4 attacks in about the

TABLE 6

*Relationship of number of relapses to months since primary attack, to mosquitoes infected, and to gametocytes per 100 white blood cells*

| No. of relapses | Cases | Months since primary attack | | Av. gametocytes per 100 w.b.c. | Av. per cent of mosquitoes infected |
|---|---|---|---|---|---|
| | | Range | Average | | |
| P* | 16 | 0 | 0.0 | 5.0 | 54.2 |
| 1 | 6 | 1–8 | 3.2 | 2.3 | 28.5 |
| 2 | 10 | 2–12 | 6.5 | 4.6 | 21.2 |
| 3 | 15 | 4–27 | 13.9 | 3.8 | 35.9 |
| 4 | 18 | 3–28 | 12.5† | 2.2 | 22.4 |
| 5 | 10 | 5–27 | 13.6 | 3.0 | 26.3 |
| 6 | 7 | 9–24 | 15.7 | 2.3 | 25.7 |
| 7 | 12 | 4–30 | 16.2 | 4.1 | 37.0 |
| 8 | 9 | 7–27 | 15.7 | 1.0 | 24.6 |
| 9 | 11 | 12–29 | 19.3 | 4.0 | 22.3 |
| 10 | 12 | 5–28 | 15.8 | 3.6 | 31.4 |
| 11 | 5 | 16–26 | 20.9 | 7.9 | 28.9 |
| 12 | 8 | 8–31 | 20.7 | 2.2 | 21.1 |
| 13 | 6 | 14–28 | 20.7 | 2.6 | 39.2 |
| 14 | 1 | 16 | 16.0 | 2.0 | 66.6 |
| 15 | 4 | 12–28 | 20.3 | 0.1 | 20.7 |
| 16 | 2 | 11–19 | 15.0 | 3.1 | 49.2 |
| 17 | 5 | 11–29 | 17.8 | 3.8 | 19.4 |
| 19 | 1 | 16 | 16.0 | 3.0 | 48.3 |
| 22 | 2 | 18–20 | 19.0 | 5.7 | 34.8 |
| 23 | 1 | 15 | 15.0 | 3.0 | 86.4 |
| 24 | 1 | 17 | 17.0 | 5.0 | 4.5 |
| 26 | 1 | 22 | 22.0 | 3.0 | 20.0 |
| Totals and averages | | | | | |
| 8.0 | 163‡ | | 15.1§ | 3.4 | 30.8 |

* Primary attack.

† Average for 17 cases. Time for one case not determined.

‡ There were 10 additional cases in which some information was indefinite.

§ Does not include 16 primary attacks.

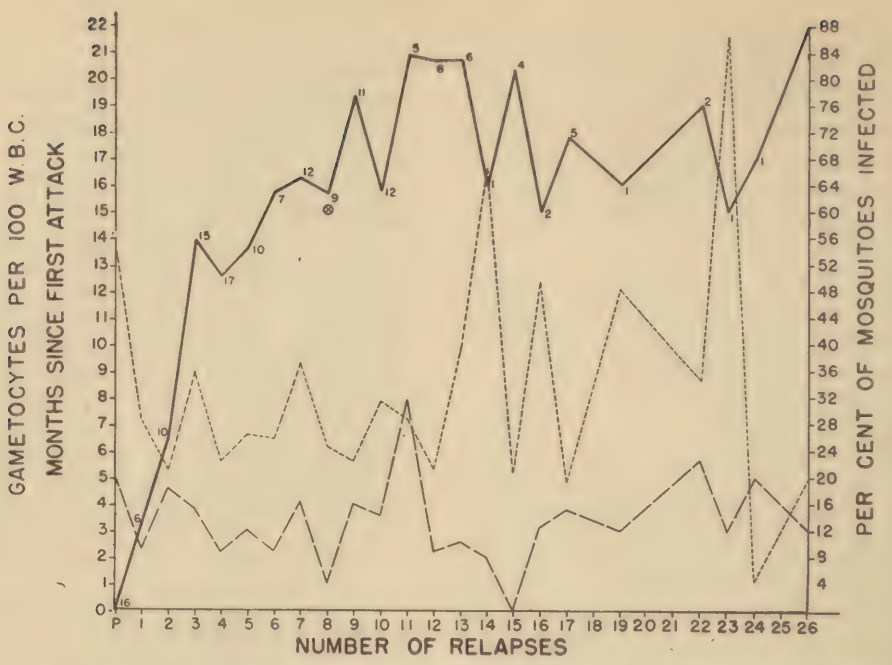



FIGURE 1. Relationship of number of relapses to months since first attack, to mosquitoes infected, and to gametocytes per 100 white blood cells in 163 cases.

——— Average number months for each relapse number (162 cases)
Numbers at points are number cases tested
- - - - - - - Per cent mosquitoes infected
— — — Gametocytes per 100 white blood cells
Delayed primary attacks = P
Average number of relapses = 8.0
Average number of months = 15.1 } ⊗

same length of time. The two extremes showed (1) 3 relapses in 9 months, and (2) 10 relapses in 5 months. The greatest duration of the disease observed was 31 months, during which time 12 relapses were experienced.

However, relapses would be of no importance if gametocytes were not produced or if mosquitoes could not be infected during the recurrences, as has been suggested by some writers.

From these data, it is apparent that gametocytes were usually found in these relapsing cases, and mosquito infections were produced, as long as the relapses occurred. The case with the most relapses (24) and the one with the longest duration of the disease (31 months) both infected mosquitoes. There did not seem to be any indication of a diminution of either the production of gametocytes or infectivity to mosquitoes as the infections persisted.

The primary attacks, of which there were 16, usually occurred several weeks after the discontinuance of suppressive drug treatment upon return to this country. The conditions were such that there was little doubt that the infections had been contracted in foreign malarious areas and not in this country. In reality, they were delayed primary attacks.

Primary cases as compared with the relapsing cases showed a higher gameto-

cyte production per 100 white blood cells (5.0 and 3.3) and a higher percentage of infectivity to mosquitoes (54.2 and 28.3). As a study has been made of the infectivity of primary infections in induced cases (table 4), these two types of primary attacks are compared as follows:

| *Primary infections* | *No. of feedings* | *Average gametocytes per 100 w.b.c.* | *Per cent infection in mosquitoes* |
|---|---|---|---|
| Neurosyphilitic patients | 112 | 5.6 | 53.9 |
| Soldiers | 16 | 5.0 | 54.2 |

It appears from these data that the primary attacks in soldiers, which had been delayed because of drug suppression, and the primary attack of infections induced in neurosyphilitic patients were quite similar in (1) the number of gametocytes produced and (2) the infectivity to *A. quadrimaculatus*. This supports the earlier evidence that the *P. vivax* malarias of foreign origin are as infective after passage through *A. quadrimaculatus* as they are in the soldiers who acquire the infection through some other vector.

### *Intensity of* P. vivax *infections in* A. quadrimaculatus

To determine the intensity of the infections, the oocysts on the guts were counted, or, in the heavy infections, estimated, and recorded under the following groupings: 1 to 9 oocysts, +; 10 to 24, ++; 25 to 99, +++; 100 and over, ++++. Sporozoites in the glands were placed in 4 intensity groupings as follows: 1 to 9, +; 10 to 99, ++; 100 to 999, +++; 1,000 and over, ++++.

There were 83 lots in which both gut and gland infections were found. The distribution of the infected mosquitoes in these lots is shown in table 7. The gut infections up to the first day of gland sporozoites and the gland sporozoites on or after the eleventh day are tabulated.

TABLE 7

*Intensity of* P. vivax *infections of foreign origin in 83 lots of* A. quadrimaculatus

| | Number of specimens under each "intensity" grouping | | | | |
|---|---|---|---|---|---|
| | + | ++ | +++ | ++++ | Totals |
| Guts with oocysts | 383 | 169 | 156 | 118 | 826 |
| Glands with sporozoites | 230 | 217 | 205 | 267 | 919 |

It is apparent from table 7 that most of the infected guts (86 per cent) had less than 100 oocysts. The sporozoites were about equally distributed among the "intensity" groupings, with 71 per cent showing fewer than 1,000 sporozoites.

There were 8 lots which showed unusually heavy infections, viz., most of the guts showed over 100 oocysts. Five of these infections were from Guadalcanal and New Guinea and 3 from the Mediterranean area.

One of the latter, v-1536-Sicily, showed the heaviest infection. Of 18 dissected specimens, the numbers of oocysts in the respective guts were: 800, 650, 500, 457, 448, 444, 400, 378, 348, 294, 270, 222, 213, 177, 161, 110, 42, and 0. The average number of oocysts for the 17 infected mosquitoes was 348.

The gametocyte counts for these 8 infected lots ranged from 0.4 to 17 per 100 white blood cells and averaged 7.5 per 100 white blood cells, as compared to 4.3 per 100 white blood cells for the total 173 counts made. However, this increase in gametocytes does not seem enough to account for such heavy infections. In other feedings, gametocyte counts as high or higher than these did not produce correspondingly heavy infections.

### *Length of sporogonous cycle of foreign* P. vivax

Fifty two infected lots of *A. quadrimaculatus* were followed by daily dissections to determine the length of the sporogonous cycle, which is defined as the elapsed time in days from the infective feeding to the first day that sporozoites are found in the salivary glands. These mosquitoes had been fed upon soldiers with relapsing malaria or upon neurosyphilitic patients with induced *P. vivax* infection of foreign origin.

The data on the length of the cycle are shown in table 8.

TABLE 8

*The length of the sporogonous cycle of foreign* P. vivax *in* A. quadrimaculatus *at temperatures between 74 and 80 F*

| Mosquito strain* | First day of sporozoites in glands of mosquitoes | | | | | | | Total lots | Average days |
|---|---|---|---|---|---|---|---|---|---|
| | 8 | 9 | 10 | 11 | 12 | 13 | 14 | | |
| Q-1 | 2 | 3 | 15 | 15 | 4 | 2 | 3 | 44 | 10.8 |
| Q-3 | 0 | 0 | 6 | 1 | 1 | 0 | 0 | 8 | 10.4 |
| Totals | 2 | 3 | 21 | 16 | 5 | 2 | 3 | 52 | 10.7 |

* Q-1 = Regular insectary colony of *A. quadrimaculatus;* Q-3 = Young colony established from *A. quadrimaculatus* caught at Longview, Tex.

Table 8 indicates that the average length of the sporogonous cycle was 10.7 days. Other infected lots dissected at 2-day intervals were not included in the table, but the length of the cycle appeared to be similar.

The shortest sporogonous cycle observed was 8 days. This was observed in 5 lots, two of which are shown in table 8. Three lots were not included in the table as daily dissections had not been done.

Three of the lots showing an 8-day sporogonous cycle were from the Mediterranean area and two were from the South Pacific.

### *Infection of mosquitoes after patient received treatment for malaria*

In 11 instances mosquitoes were applied after therapy had been started. Ten of the patients had relapsing *P. vivax* infections. Of these ten, 8 had received atabrine in varying amounts for periods ranging from 5 hours to several days. Seven of these 8 cases produced infections in mosquitoes. Of the other two patients with *P. vivax* infection, one had been treated with quinine and the other with quinine and plasmochin; both produced infections in mosquitoes. One patient with *P. falciparum* infection had been given an unknown quantity of atabrine and did not produce infections in the mosquitoes.

Nine of the 11 patients produced infections in mosquitoes after having received some antimalarial drug. This again shows that carriers of these foreign malarias can spread the disease even after having received some treatment.

### *Development of* P. vivax *infection in* A. quadrimaculatus *at outside temperatures*

Experiments were set up to determine how these foreign malarias would develop at outside temperatures in Longview, Tex.

After feeding, the mosquitoes were put into a cage placed under the laboratory, the floor of which was about 4 feet above the ground. The cage was put into an inverted trunk locker and was protected from drafts by curtains of unbleached muslin. Conditions in the cage were thought to approximate roughly those in natural daytime resting places usually selected by *A. quadrimaculatus.*

The temperature readings shown were those routinely taken by the Harmon General Hospital at the fire house which is located about one-half mile from the laboratory. A comparison of readings

taken under the laboratory and at the fire house showed the minimum readings to be about the same, but the maximum readings, during the warmer days, were higher at the fire house, amounting to 14 degrees difference on the hottest day, which was 98 F at the fire house. The outside mean temperatures shown were simply an average of the highest and lowest temperatures during the 24-hour period.

The first experiment was started April 11th, 1945, and consisted of 5 feedings made during the next 17 days. Part of the mosquitoes were put under the laboratory and part kept in the insectary at controlled temperatures. Both the Q–1 and Q–3 strains of *A. quadrimaculatus* were used in this experiment. In the second experiment, all the mosquitoes were placed under the laboratory. Only the Q–1 strains of *A. quadrimaculatus* was used.

The data for these experiments are shown in table 9.

In the first experiment with the mosquitoes kept outdoors, the development of the oocysts proceeded normally for about 10 days; development of oocysts was slower in mosquitoes kept outdoors than in those kept in the insectary.

About the tenth day many of the oocysts in those kept outdoors began to show vacuoles, which increased in size until the oocyst degenerated, with older oocysts showing almost no protoplasm. Other oocysts developed sporoblasts and sporozoites, but comparatively slowly. Mature oocysts rarely appeared before the fourteenth day. During the period from the sixth day to the development of mature oocysts, the number of oocysts in the gut decreased considerably in some lots of mosquitoes. A batch which showed heavy gut infections on the sixth day might show only a light gut infection on the tenth or twelfth day, and about 50 per cent of the oocysts might be abnormal or degenerating.

TABLE 9

*Development of* P. vivax *infections of foreign origin in* A. quadrimaculatus *at outside temperatures*

| | Mosquitoes kept | | |
|---|---|---|---|
| | Insectary | Outside | Outside only |
| | First experiment* | | Second experiment† |
| Temperatures:‡ | | | |
| Maximum av. | 80 | 80.9 | 81.8 |
| Minimum av. | 75 | 58.6 | 59.7 |
| Mean av. | 77 | 69.7 | 70.8 |
| Mosquitoes: | | | |
| Dissected | 223 | 255 | 402 |
| Infected | 121 | 146 | 274 |
| Per cent infected | 54.3 | 57.3 | 68.2 |
| Sporogonous cycle: | | | |
| Range (*days*) | 10–12 | 15–23 | 10–13 |
| Average | 10.8 | 16.6 | 11.5 |

* First experiment consisted of 5 feedings during period of April 11th to April 28th, 1945.

† Second experiment consisted of 10 feedings during period of May 6th to May 18th, 1945.

‡ Outside temperatures taken at Harmon General Hospital fire house about 0.5 mile away from place where cages were kept.

As seen in table 9, the sporozoites began to appear in the glands much later in the outdoor mosquitoes than in those kept in the insectary, the average length of the sporogenous cycle being about 6 days longer for the former.

Infected mosquitoes from 3 lots kept at outside conditions were applied to neurosyphilitic patients and transmission was successful in all 3 cases. These transmissions represented infections from New Guinea, Sicily, and North Africa.

In the second experiment, the infections in the outdoor mosquitoes developed faster than in the first experiment. This was undoubtedly due to the warmer temperatures prevailing during

the day although this was not accurately reflected in the mean temperatures shown. There was less tendency for the oocysts to show degeneration. The average duration of the sporogonous cycle was only slightly longer than the average duration in the insectary.

One of these infected lots, derived from an infection acquired in New Guinea was applied to a neurosyphilitic patient, and transmission of infection was effected.

Sporozoites which appeared to be degenerate were found in the infected lots of both the first and second experiments and will be discussed later. These degenerate forms appeared both in the mosquitoes kept in the insectary and in those kept outside.

In table 9 (first experiment) it is seen that the infection rate was about the same in both the mosquitoes kept in the insectary and those kept at outside conditions.

The above observations show that malarias of foreign origin developed in *A. quadrimaculatus* mosquitoes under temperature conditions approximating those in nature in Texas during April and May. The rate of infection was similar in the mosquitoes kept outdoors and in the insectary.

### *The appearance of abnormal sporozoites*

At the Texas laboratory during the spring of 1945 sporozoites were seen which were abnormal in morphology; these were seen only in the glands. The sporozoites were bent in the middle to form a "v." The nucleus was enlarged to varying degrees and was located in the angle of the bend. The shape of most was similar to those described by Barber (1936) as appearing in *Anopheles elutus*. Some resembled the forms that he described from *A. maculipennis*, particularly in having a considerable enlargement at the angle. Barber designated such forms as degenerate.

Abnormal forms were found both in the mosquitoes kept in the insectary and in those kept outdoors. From the latter, attempts to transmit 3 different infections to 4 patients were made and all were successful. The prepatent and incubation periods of the resulting infections were within the normal range. In these transmission attempts, practically all of the sporozoites were of the abnormal shape, a prolonged search being necessary to find any that appeared normal.

The development of the abnormal shape seemed to occur after the sporozoites left the oocyst. When mature oocysts were crushed by pressure, sporozoites that appeared normal were released.

Definite localization of sporozoites in different lobes of the gland according to the normal state or degree of degeneration such as described by Barber was not observed. It was found, however, that there was much variation in the numbers of sporozoites in different lobes.

The origin of the infection did not seem to be relevant, as infections from Sicily, North Africa, and New Guinea all showed the abnormal sporozoites. Also, as shown above, it occurred both at controlled insectary and fluctuating outside temperatures.

The only factor which could be found consistently associated with the abnormal sporozoites was a fungus infection in the mosquitoes. During the spring of 1945, the mosquito colony became heavily parasitized with a fungus, the species of which could not be identified by us or by the bacteriologist at the hospital. The fungus appeared to cause a heavy mortality among the infected mosquitoes, particularly those kept in

the insectary. Those kept outside showed less infestation and a lower mortality.

The fungus often caused the abdomen of the mosquito to become swollen. It invaded the thorax and was frequently found in close association with the glands when the latter were dissected.

The presence of fungus did not seem to alter the susceptibility of the mosquito to infection with malaria. Some lots were highly infected and others were not.

Barber suggested that the degeneration of sporozoites may help to explain the relative inability of *A. superpictus* to transmit malaria in some parts of Macedonia. Our transmission attempts were successful. This then would indicate that: (1) the abnormalities found in *A. quadrimaculatus* were probably different from those shown by Barber; (2) that the viability of the sporozoites was not affected as he suggested for *A. superpictus;* or (3) that there were enough normal sporozoites in the *A. quadrimaculatus* to produce the infections within the normal incubation time. The last mentioned seems the most unlikely of the 3 suggestions, as it was difficult to find normal sporozoites in the glands of the mosquitoes which transmitted infections.

### *Infectivity of* P. vivax *malaria of foreign origin to various species of southern anophelines*

Three species of anophelines, other than *A. quadrimaculatus,* were tested for their susceptibility to foreign malarias. They were always fed simultaneously with *A. quadrimaculatus.*

*A. albimanus* and *A. pseudopunctipennis pseudopunctipennis* were collected in the Brownsville, Tex., area and were tested at the Longview, Tex., laboratory. Specimens of *A. punctipennis* were collected in South Carolina and tested at Swannanoa, N. C. Usually the first few generations removed from the field-caught adults were used.

These species were fed upon soldiers with relapsing malaria and neurosyphilitic patients with induced malaria. In these comparative experiments, all mosquitoes which did not take a blood meal were killed immediately. As is shown elsewhere, relapsing malaria in the returned soldiers and malaria induced in neurosyphilitic patients give comparable infections in *A. quadrimaculatus.* No breakdown according to these two types of patients will be given in the results for the other mosquito species.

The results are shown in table 10. *A. walkeri* were also tried but too few were dissected to permit a valid comparison.

In 4 of the above feedings (B.L., L.A., C.T., C.T.), *A. quadrimaculatus, A. pseudopunctipennis pseudopunctipennis,* and *A. albimanus* were fed simultaneously. The percentages of infections resulting were: *A. quadrimaculatus,* 69; *A. pseudopunctipennis pseudopunctipennis,* 16; *A. albimanus,* 0.

As shown in table 10, *A. punctipennis* and *A. quadrimaculatus* were more than twice as susceptible as *A. pseudopunctipennis pseudopunctipennis* and about 50 times as susceptible as *A. albimanus.* Theoretically one might draw up a numerical evaluation showing what percentage of the different species might be expected to become infected in the same situation where the most susceptible species showed a 100 per cent infectivity, as follows:

| | |
|---|---|
| *A. punctipennis* | 100 |
| *A. quadrimaculatus* | 98 |
| *A. pseudopunctipennis pseudopunctipennis* | 40 |
| *A. albimanus* | 2 |

In addition to determining the relative susceptibility of the above 4 species

TABLE 10

*The infectivity of* P. vivax *infections of foreign origin to 3 mosquito species compared with* A. quadrimaculatus

| Infection number | Patient | Gameto-cytes per 100 w.b.c. | Control species mosquitoes | | | Test species mosquitoes | | |
|---|---|---|---|---|---|---|---|---|
| | | | Inf. | Diss. | Per cent inf. | Inf. | Diss. | Per cent inf. |
| | | | *A. quadrimaculatus* | | | *A. punctipennis* | | |
| 1527-NG* | G. P. | 3.0 | 24 | 27 | 88.8 | 10 | 10 | 100.0 |
| 1528-NG | W. T. | 3.0 | 34 | 35 | 97.1 | 21 | 21 | 100.0 |
| 1532-NG | R. S. | 0.0 | 18 | 21 | 85.7 | 12 | 15 | 80.0 |
| Totals and averages | 3 | 2.0 | 76 | 83 | 91.5 | 43 | 46 | 93.4 |
| | | | *A. quadrimaculatus* | | | *A. pseudopunctipennis pseudopunctipennis* | | |
| 3-G | G. J. | 7.7 | 27 | 38 | 71.0 | 5 | 16 | 31.2 |
| 1027-NG | B. L. | 3.0 | 27 | 60 | 45.0 | 4 | 10 | 40.0 |
| 1027-NG | R. S. | 4.0 | 27 | 60 | 45.0 | 1 | 3 | 33.3 |
| 1027-NG | B. L. | 9.0 | 21 | 30 | 70.0 | 3 | 10 | 30.0 |
| 1027-NG | L. A. | 4.0 | 7 | 35 | 20.0 | 0 | 5 | 0.0 |
| 1027-NG | C. T. | 5.0 | 33 | 44 | 75.0 | 0 | 5 | 0.0 |
| 1027-NG | C. T. | 9.0 | 48 | 50 | 96.0 | 2 | 12 | 16.7 |
| Totals and averages | 7 | 6.0 | 190 | 317 | 59.9 | 15 | 61 | 24.6 |
| | | | *A. quadrimaculatus* | | | *A. albimanus* | | |
| 1027-NG | B. L. | 9.0 | 21 | 30 | 70.0 | 0 | 8 | 0.0 |
| 1027-NG | L. A. | 4.0 | 7 | 35 | 20.0 | 0 | 20 | 0.0 |
| 1027-NG | C. T. | 5.0 | 33 | 44 | 75.0 | 0 | 22 | 0.0 |
| 1027-NG | C. T. | 9.0 | 48 | 50 | 96.0 | 0 | 56 | 0.0 |
| 1027-NG | J. H. | 1.0 | 32 | 45 | 71.1 | 0 | 48 | 0.0 |
| 1027-NG | J. O. | 4.0 | 34 | 42 | 81.0 | 1 | 53 | 1.9 |
| 1027-NG | J. H. | 6.0 | 31 | 37 | 84.8 | 2 | 43 | 4.7 |
| 1031-NG | H. M. | 4.0 | 29 | 30 | 96.7 | 0 | 52 | 0.0 |
| 1027-NG | M. T. | 3.0 | 24 | 53 | 45.3 | 1 | 53 | 1.9 |
| 1027-NG | H. B. | 2.0 | 24 | 42 | 57.1 | 0 | 37 | 0.0 |
| 1027-NG | R. R. | 3.0 | 24 | 38 | 63.2 | 2 | 33 | 6.1 |
| Totals and averages | 11 | 5.6 | 307 | 446 | 68.8 | 6 | 425 | 1.4 |

* NG = New Guinea; G = Guadalcanal.

by comparing the percentages infected when fed at the same time, another comparison can be made by the number of oocysts found per gut among the infected mosquitoes.

*A. punctipennis* had about the same number of oocysts per gut as did *A. quadrimaculatus*. *A. pseudopunctipennis pseudopunctipennis* and *A. albimanus* had too few infected guts to justify a percentage comparison with *A. quadrimaculatus* but they consistently had fewer oocysts per gut than the latter species.

## Discussion

As indicated in the foregoing data, the southern vector of malaria, *A. quadrimaculatus*, is a favorable vector of malarias of foreign origin. Mosquitoes became infected from more than one-half (61 per cent) of the 165 cases. Not only did the first attempts at infecting usually succeed, but after several (5 in some instances) man-mosquito passages, infectivity could still be demonstrated, although *A. quadrimaculatus* was a new vector species.

The malarias tested came from widely scattered areas of the world, and, as was to be expected, some of them definitely seemed to be strains distinct from strains found in this country (Ehrman et al, 1945).

In the breakdown of the foreign malarias according to the areas from which they originated, it was found that there did not appear to be much difference in regard to infectivity between the malarias from the South Pacific and those from the Mediterranean.

The cases studied from the other areas, viz., Carribean, Burma, and Liberia, all showed some infectivity for mosquitoes, although the data were not sufficient to draw a comparison on the above point. Nothing was found which would indicate that any great difference might be expected to exist.

The finding that patients can continue to infect mosquitoes as long as they relapse makes the problem of control a continuing one. At present it is not known how long soldiers with these malarias of foreign origin will relapse. In the sample that we had for our work, the soldiers had an average of 8 relapses each over a period (average) of 15 months. Some of the soldiers were in the third year of their infection. The magnitude of the problem cannot be measured until information has been secured regarding how long these relapses will occur and the number of soldiers returning with infections.

The opinion has been expressed that a patient receiving any malarial therapy is not infective to mosquitoes. While this is generally true after the patient has received a substantial amount of treatment, the results presented in this paper support other results indicating that patients may be infective for a few days after therapy is started.

Most of the infections reported herein were incubated at controlled temperatures which were supposed to be near the optimum for the species. However, the placing of some of the potentially infected mosquitoes at outside temperatures (at the Texas laboratory) indicates that these foreign malarias are not restricted in their development to a controlled temperature of between 74 and 80 F but can develop at outside temperatures under conditions which approximate those at which *A. quadrimaculatus* are ordinarily found.

Not only were the malarias of foreign origin shown to be infective to *A. quadrimaculatus* (the most important malaria vector in the southern United States) but they were also infective to *A. punctipennis* at a rate similar to that of *A.*

*quadrimaculatus.* Until the present, *A. punctipennis* has not been considered a good vector under natural conditions. However, it is felt that this concept is not supported by adequate comparative field tests of the two species. Until such time as these tests are made, it would not seem wise to rule out *A. punctipennis* as an important vector in this country of these foreign malarias.

In passing, it should be pointed out that in some lots heavy infections were produced in the mosquito. The evidence was not sufficient to indicate that these heavy infections were due to particularly virulent strains. The possible existence of such strains, however, should be kept in mind, especially in the case of outbreaks due to foreign malarias.

In the laboratory, the behavior of *P. vivax* malarias of foreign origin is similar to the malarias caused by the St. Elizabeth's strain. Comparable information is not available on indigenous malarias under natural conditions. On the basis of laboratory evidence it appears that these foreign malarias will be transmitted as readily as our own.

## Summary and conclusions

1. The infectivity of malarias of foreign origin relapsing in returned soldiers to anophelines of the southern United States was investigated. A total of 173 lots of *Anopheles quadrimaculatus* was fed on soldiers with relapsing *Plasmodium vivax* infection. The origins of the infections were: South Pacific, 117; Mediterranean, 40; Carribean, 6; Liberia, 1; Burma, 1.

2. Malarias from each of the above areas infected *A. quadrimaculatus;* 61 per cent of the cases infected mosquitoes. The rate of infection in the total 6,247 mosquitoes dissected was 30.8 per cent.

3. *A. quadrimaculatus* successfully transmitted these infections to man. These foreign malarias induced in neurosyphilitic patients were also infective to *A. quadrimaculatus,* and continued to be so through several serial transfers both by mosquito bites and blood inoculations.

4. The average gametocyte count in the relapsing soldiers was 3.4 per 100 white blood cells. In general, an increase in gametocytes resulted in an increase in infection with the sharpest increase occurring at one gametocyte per 100 white blood cells.

5. Gametocytes were produced and mosquitoes were infected as long as relapses occurred. The case with the most relapses (24) and the case with the longest duration of the disease (31 months) both infected mosquitoes.

6. Fourteen per cent of the infected mosquito guts had over 100 oocysts each. Of the infected glands, 29 per cent showed over 1,000 sporozoites.

7. The average length of the sporogonous cycle of *P. vivax* in *A. quadrimaculatus* was 10.7 days when incubated at 74 to 80 F; the shortest was 8 days (found in 3 cases from the Mediterranean and 2 from the South Pacific).

8. Of 10 cases of *P. vivax* infection in which antimalarial therapy had been begun, 9 produced infections in *A. quadrimaculatus.* One *P. falciparum* infection failed to infect a mosquito.

9. Mosquitoes kept at outdoor temperatures in Texas during April and May developed infections at about the same rate as those kept in the insectary. During April the infections developed more slowly outdoors.

10. Abnormal sporozoites appeared in some of the mosquito glands. These were correlated with a fungus concomitantly infecting the mosquitoes.

11. *A. quadrimaculatus* recently colonized from nature and mosquitoes of the same species from a long established

colony showed similar rates of infection when fed simultaneously.

12. *A. punctipennis, A. pseudopunctipennis pseudopunctipennis,* and *A. albimanus* also became infected by the malarias of foreign origin. A theoretical numerical evaluation of the relative susceptibility under the same situation, when the most susceptible showed a 100 per cent infectivity, would be: *A. punctipennis,* 100; *A. quadrimaculatus,* 98; *A. pseudopunctipennis pseudopunctipennis,* 40; *A. albimanus,* 2.

13. Of the two cases of *P. falciparum* infection encountered, one infected *A. quadrimaculatus.*

14. The malarias of foreign origin studied readily infected *A. quadrimaculatus* and were transmitted by this vector.

## References


Barber, M. A.
1936 Degeneration of sporozoites of the malaria parasite in anopheline mosquitoes in nature and its relation to the transmission of malaria. Amer. Jour. Hyg., *24:* 45–56.

Boyd, M. F.
1939 On the susceptibility of *Anopheles quadrimaculatus* to *Plasmodium vivax* after prolonged insectary cultivation. Amer. Jour. Trop. Med., *19:* 593–594.

Burgess, R. W., and Young, M. D.
1944 Methods of handling and feeding *Anopheles quadrimaculatus* Say upon malarious patients. Jour. Nat. Mal. Soc., *3:* 241–247.

Ehrman, F. C., Ellis, J. M., and Young, M. D.
1945 *Plasmodium vivax* Chesson strain. Science, *101:* 377.

Moore, J. A., Young, M. D., Hardman, N. F., and Stubbs, T. H.
1945 Studies on imported malarias: 2. Ability of California anophelines to transmit malarias of foreign origin and other considerations. Jour. Nat. Mal. Soc., *4:* 307–329.

Young, M. D., Ellis, J. M., and Stubbs, T. H.
1946 Studies on imported malarias: 5. Transmission of foreign *Plasmodium vivax* by *Anopheles quadrimaculatus.* Amer. Jour. Trop. Med. (in press).

Young, M. D., Stubbs, T. H., Moore, J. A., Ehrman, F. C., Hardman, N. F., Ellis, J. M., and Burgess, R. W.
1945 Studies on imported malarias: 1. Ability of domestic mosquitoes to transmit *vivax* malarias of foreign origin. Jour. Nat. Mal. Soc., *4:* 127–131.

Reprinted from THE AMERICAN JOURNAL OF TROPICAL MEDICINE
Vol. 26, No. 4, July, 1946

# STUDIES ON IMPORTED MALARIAS

## 5. TRANSMISSION OF FOREIGN PLASMODIUM VIVAX BY ANOPHELES QUADRIMACULATUS[1]

MARTIN D. YOUNG,[2] JOHN M. ELLIS[3] AND TRAWICK H. STUBBS[4]

### INTRODUCTION

It has been previously shown (Young, et al., 1946) that the mosquitoes of the southern United States were susceptible to foreign malarias relapsing in returned troops and that these malarias developed to the infective stage in the insects. A preliminary report (Young, et al., 1945) indicated that the infected mosquitoes could successfully transmit the foreign malarias.

This report will present the detailed observations on the ability of *Anopheles quadrimaculatus* to transmit various foreign malarias to white and Negro patients. The patients were neurosyphilitics, with the exception of 14 white men who were volunteers on a drug testing program. The infections in these mosquitoes originated from the relapsing cases reported by Young, et al. (1946).

### METHODS

Mosquitoes from a lot proven to have sporozoites in the salivary glands were applied to the patients selected. The mosquitoes were dissected after feeding and the number with sporozoites determined.

Starting several days after the biting day, blood smears were made daily and examined for parasites. Temperature readings were made at regular intervals, usually every four hours during the afebrile state and hourly during fever periods.

An effort was made to determine whether the patient had had malaria previously, either natural or induced.

### OBSERVATIONS

*Total Transmission Attempts.* Transmission was attempted on 186 patients as shown in table 1.

[1] Contribution from the Imported Malaria Studies program of the Office of Malaria Investigations, National Institute of Health, and the Office of Malaria Control in War Areas.

The following hospitals cooperated by making neurosyphilitic patients available: Harmon General, Moore General, South Carolina State, Milledgeville (Ga.) State, North Carolina State at Morganton and Raleigh, and the University Hospital at Augusta, Ga. To these, and especially to Harmon General, Moore General, and the South Carolina State Hospitals, which also furnished laboratory quarters, we express appreciation. We are indebted also to the Office of the Surgeon General, U. S. Army, whose active interest made the program possible.

[2] Sanitarian (R) U. S. Public Health Service
[3] P. A. Sanitarian (R) U. S. Public Health Service
[4] P. A. Surgeon, U. S. Public Health Service

Read at the annual meeting of the American Society of Tropical Medicine, Cincinnati, Ohio, November 13, 1945.

It is seen from table 1 that most of the white patients (92.1 per cent) developed malaria after the first transmission attempt and that 94.7 per cent became infected after one or more attempts. The Negroes became infected at a much lower rate after the first trial (28.6 per cent) and after multiple trials (31.4 per cent).

Of the 19 patients (8 white and 11 Negro) upon whom additional transmission attempts were made, the number of such trials were as follows:

4 (white—3; Negro—1) patients were infected on second trial.
1 (white—1; Negro—0) patient was infected on third trial.
6 (white—2; Negro—4) patients were not infected on second trial.
8 (white—2; Negro—6) patients were not infected on third trial.

TABLE 1

*Summary of all attempts to transmit foreign* P. vivax *to 186 patients*

| | FIRST TRIAL | SUBSEQUENT TRIALS | TOTAL |
|---|---|---|---|
| White patients | | | |
| Tried | 151 | 8* | 151 |
| Infected | 139 | 4* | 143 |
| % infected | 92.1 | 50.0 | 94.7 |
| Negro patients | | | |
| Tried | 35 | 11* | 35 |
| Infected | 10 | 1* | 11 |
| % infected | 31.4 | 9.1 | 31.4 |
| Total patients | | | |
| Tried | 186 | 19* | 186 |
| Infected | 149 | 5* | 154 |
| % infected | 80.1 | 26.3 | 77.4 |

* Failures on first trial.

It is apparent that while additional trials infected 4 of the 8 white patients who failed on the first attempt, only one out of 11 Negroes was infected by repeated trials.

*Transmission of Foreign P. vivax According to Origin of the Malarias.* The origins of the malarias transmitted are shown in table 2.

There was less difference in the transmission rates of the malarias from the Pacific and those from the Mediterranean areas than between different places within each of these areas.

*Transmission of Different Strains.* For convenience, each relapsing case of malaria studied in returned troops was arbitrarily designated as a different strain and assigned a number.

In the transmission attempts, 43 strains were involved of which 34 were transmitted. These data are presented in table 3.

Most of the strains from every area infected white patients. All of the 11

TABLE 2

*Transmission of foreign* vivax *malarias by* A. quadrimaculatus *to white and negro patients. Arranged by origin of malaria. (First inoculations only. No reinoculations included)*

| ORIGIN OF STRAIN | PATIENTS | | | | | |
|---|---|---|---|---|---|---|
| | White | | | Negro | | |
| | Tried | Infected | Per cent infected | Tried | Infected | Per cent infected |
| Guadalcanal | 37 | 31 | 83.8 | 9 | 3 | 33.3 |
| New Guinea | 80 | 76 | 95.0 | 9 | 1 | 11.1 |
| Total Pacific | 117 | 107 | 91.5 | 18 | 4 | 22.2 |
| North Africa | 7 | 6 | 85.7 | 4 | 1 | 25.0 |
| Sicily & Italy | 26 | 25 | 96.2 | 13 | 5 | 38.5 |
| Total Mediterranean | 33 | 31 | 93.9 | 17 | 6 | 35.3 |
| Burma | 1 | 1 | 100.0 | 0 | 0 | 0.0 |
| Total | 151 | 139 | 92.1 | 35 | 10 | 28.6 |

TABLE 3

*Transmission of different strains of foreign* P. vivax *by* A. quadrimaculatus

| ORIGIN OF STRAINS | MALARIA STRAINS TRANSMITTED TO: | | | | | |
|---|---|---|---|---|---|---|
| | White Patients | | Negro Patients | | Total Strains* | |
| | Attempts | Successes | Attempts | Successes | Attempts | Successes |
| Guadalcanal | 15 | 11 | 6 | 3 | 17 | 11 |
| New Guinea | 11 | 11 | 5 | 1 | 11 | 11 |
| Total Pacific | 26 | 22 | 11 | 4 | 28 | 22 |
| North Africa | 6 | 5 | 2 | 1 | 8 | 6 |
| Sicily & Italy | 5 | 4 | 3 | 3 | 6 | 5 |
| Total Mediterranean | 11 | 9 | 5 | 4 | 14 | 11 |
| Burma | 1 | 1 | 0 | 0 | 1 | 1 |
| Total | 38 | 32 | 16 | 8 | 43 | 34 |

* As some of the same strains were tried in both white and Negro patients, the totals are not necessarily the same as the addition of the numbers under these two categories.

New Guinea strains produced infections as did the one Burma strain. Fewer of the various strains infected Negroes than was the case with the white paretics.

This, together with the total transmission rates shown in table 2, indicates that the Negro's resistance to *P. vivax* is not limited to strains from a particular area but is a general resistance to the species as found in widely separated regions.

*Comparison of the Transmission of Several Strains of Foreign P. vivax to Both White and Negro Neurosyphilitic Patients.* To study further the transmission

TABLE 4

*Comparison of Transmission of Several Strains of Foreign* P. vivax *to Both White and Negro Neurosyphilitic Patients*

| STRAIN NUMBER | WHITE PATIENTS | | NEGRO PATIENTS | |
|---|---|---|---|---|
| | Attempts | Successes | Attempts | Successes |
| 1005G | 6 | 6 | 1 | 0 |
| 1012G | 1 | 1 | 1 | 1 |
| 1019G | 3 | 2 | 3 | 1 |
| 1023G | 1 | 1 | 1 | 1 |
| 1017NG | 1 | 1 | 1 | 0 |
| 1027NG | 52 | 49 | 4 | 1 |
| 1030NG | 4 | 4 | 2 | 0 |
| 1033NG | 3 | 3 | 1 | 0 |
| 1034NG | 2 | 2 | 1 | 0 |
| 1031Si | 21 | 21 | 10* | 2 |
| 1037Si | 2 | 2 | 1 | 1 |
| Total | 96 | 92 | 26 | 7 |
| Per Cent | | 95.8 | | 26.9 |

* One lot fed on Negroes but not on whites. Three patients involved—all failures.
G—Guadalcanal; NG—New Guinea; Si—Sicily.

TABLE 5

*The effect of a previous infection of* P. vivax *malaria (St. Elizabeth strain) upon subsequen inoculation with foreign strains. White patients*

| PATIENTS | P. VIVAX (ST. ELIZABETH STRAIN) NUMBER PAROXYSMS | FOREIGN P. VIVAX* NUMBER PAROXYSMS |
|---|---|---|
| J. M. D. | 2 | 20† |
| A. C. | 9‡ | 9 |
| L. G. | 13‡ | 11† |

* Three Pacific strains used—78, 90, and 94.
† Terminated by drug. All others self-terminated.
‡ Infection by blood inoculation. All others by mosquitoes.

rates to white and Negro patients, comparative tests were made using the same strains. With one exception, the same lot of infected mosquitoes was applied to both white and Negro patients at the same time. The number of infected mosquitoes biting each type of patient was about the same. These data are shown in table 4.

These results show the difference in susceptibility between Negroes and whites

to foreign *P. vivax* even more strikingly. The ratio of successful transmissions in white patients as compared with Negroes was 3.6 to 1.

*Effects of Previous Infections Upon the Development of Foreign P. vivax.* Three white neurosyphilitic patients who had just previously experienced a primary symptomatic infection of the St. Elizabeth strain of P. *vivax* were reinoculated by mosquitoes infected with foreign *P. vivax*. These reinoculations were given between 18 and 42 days after the last paroxysms with St. Elizabeth *P. vivax*. These data are presented in table 5.

Without treatment, the St. Elizabeth *P.vivax* infections had ceased to produce fevers and the parasite count had dropped to a low level indicating the production of an immunity against that strain. Such immunity did not prevent the foreign malarias from producing infections, which in 2 instances had to be terminated by drugs.

## DISCUSSION

It appears from the data presented that white neurosyphilitics in this country will readily develop infections of foreign *P. vivax* when bitten by infected mosquitoes. The malarias originated from widely separated areas of the world and all showed a high rate of infectivity.

With Negro neurosyphilitic patients, the results were quite different. Of the total transmission attempts with all strains, the Negroes showed a much lower susceptibility. In a series in which the Negro and white patients were tested with the same strains, the difference was even greater, the Negroes being infected in only 26.9 per cent of the cases as against 95.8 per cent for the white patients.

It has been demonstrated repeatedly in this laboratory and by others (Boyd, M. F. and Stratman-Thomas, W. K., 1933) that *P. vivax* malaria usually cannot be induced in southern Negro neurosyphilitic patients, either by infected blood or mosquitoes.

Residence in a malarious area has been thought by some to be related to the resistance of Negroes to *vivax* infections. Among the Negro patients reported here, 29 were questioned as to previous residence. Five of 22 from the Southeast developed infections, including one with a previous malaria history. Four of the 7 from outside this area became infected. Even if these inadequate data were representative, there would still be a large unexplained difference between the two races.

The possibility of the presence of neurosyphilis affecting the susceptibility of Negroes to *P. vivax* malaria and not affecting white patients does not seem likely. Neurosyphilis does not seem to exert such a differential effect against *P. malariae* or *P. falciparum*.

Should the Negroes have a true racial immunity to foreign *P. vivax* , the possibility of the spread of these malarias in this country would be greatly lessened.

No great difference was found by us in the transmissibility of the *P. vivax* strain from widely separated areas, such as the South Pacific and Mediterranean. It appears that most of the imported foreign strains can be readi y transmitted by *A. quadrimaculatus*.

SUMMARY AND CONCLUSIONS

1. One or more attempts were made to transmit foreign *Plasmodium vivax* by *Anopheles quadrimaculatus* to each of 186 men. Of the 151 white patients, 94.7 per cent were infected. Of the 35 Negro patients, 31.4 per cent were infected. On the first attempt, 92.1 per cent of the white patients and 28.6 per cent of the Negroes became infected.

2. When the same strains were tested against both, white patients were more readily infected than Negro patients in a ratio of 3.6 to 1.

3. The malarias tried originated from widely separated areas of the world and all showed a high rate of infectivity to white patients. The Negro seemed to have a general resistance to *P. vivax* from all areas rather than to strains from particular areas only.

4. In 3 white cases, a recent infection with the St. Elizabeth strain of *P. vivax* did not prevent the development of foreign strains of *P. vivax*. This indicated that little or no immunity was gained from the former strain. Should this be true for all American strains, the white population of this country could be considered as non-immune to foreign *P. vivax* malarias.

REFERENCES

Boyd, M. F. and Stratman-Thomas, W. K. 1933. Studies on Benign Tertian Malaria. 4. On the Refractoriness of Negroes to Inoculation with *Plasmodium vivax*. Am. Jr. Hyg. **18**(2): 485–489.

Young, M. D., Stubbs, T. H., Moore, J. A., Ehrman, F. C., Hardman, N. F., Ellis, J. M., and Burgess, R. W. 1945. Studies on Imported Malarias. 1. Ability of Domestic Mosquitoes to Transmit *vivax* Malaria of Foreign Origin. Jour. Nat. Mal. Soc. **IV(2)**: 127–131.

Young, M. D., Stubbs, T. H., Ellis, J. M., Burgess, R. W., and Eyles, D. E. 1946. Studies on Imported Malarias. 4. The Infectivity of Malarias of Foreign Origin to Anophelines of the Southern United States. Am. Jr. Hyg. **43**(3): 326–341.

Reprinted from THE AMERICAN JOURNAL OF TROPICAL MEDICINE
Vol. 27, No. 5, September, 1947

# SOME CHARACTERISTICS OF FOREIGN VIVAX MALARIA INDUCED IN NEUROSYPHILITIC PATIENTS[1, 2]

MARTIN D. YOUNG, JOHN M. ELLIS AND TRAWICK H. STUBBS[3]

Received for publication March 26, 1947

During the program of studying foreign malaria imported by returning service men, it has been shown that these strains are infective to and can be transmitted by our native malaria vectors (7, 9, and 11). Neurosyphilitic patients were inoculated therapeutically and from these patients certain data were obtained bearing upon the host-parasite relationship. These observations form the basis of the present report.

## METHODS

Practically all patients treated were service men. While under malaria-therapy, oral temperatures were taken every 4 hours except during fevers when temperatures were taken hourly or even more frequently. During the fever, patients received the usual symptomatic care accorded those undergoing malaria-therapy.

Most of the infections were transmitted by bites of infected mosquitoes which were mainly *Anopheles quadrimaculatus;* some infections were induced by blood transfer. Blood smears were taken at least once daily. Density counts of malaria parasites were made by the Earle-Perez (2) method with about 0.1 cmm. of blood examined as a minimum.

Although a total of 27 strains was employed, the principal strains of malaria used were four from the Pacific area and one from the Mediterranean area. One of the Pacific strains used (v-1027-NG) apparently originated in New Guinea and has been designated the "Chesson" strain (3). This strain has been used extensively in the investigation of new anti-malarial drugs.

## OBSERVATIONS

*Prepatent and Incubation Periods (Mosquito Induced Malaria).* Usually from 5 to 10 infected mosquitoes bit the recipient patient, but in a few cases the number varied from 1 to 22.

Data were available in 123 cases for both the prepatent and incubation

[1] This is the sixth in a series of reports on imported malarias. The complete title of this report reads, "Studies on Imported Malarias. 6. Some Characteristics of Foreign vivax Malarias Induced in Neurosyphilitic Patients".

[2] Contribution from the Imported Malaria Studies program of the Office of Malaria Investigations, National Institute of Health, and the Office of Malaria Control in War Areas, United States Public Health Service, Columbia, S. C.

The Harmon General Hospital and the South Carolina State Hospital furnished laboratory space and made possible the securing of the information upon the induced infections. To the staffs of these hospitals we express our appreciation as well as to the Office of the Surgeon General, United States Army, whose active interest made the program possible.

[3] Now Assistant Dean, Emory University Medical School.

periods. These data are given in table 1. It is apparent from the table that the Mediterranean strains of *P. vivax* gave rise to infections in which the developmental period was shorter than in strains of malarias from the Pacific or Burma area. To test this further, the strains were compared statistically, the most frequently used strains from the Pacific and Mediterranean areas being chosen

TABLE 1

*Prepatent and Incubation Periods of Foreign P. vivax Induced in Neurosyphilitic Patients*

Transmission by *A. quadrimaculatus*. Only first inoculations included. Malarias arranged by areas of origin

| AREA OF ORIGIN | WHITE PATIENTS | | | | NEGRO PATIENTS | | | | TOTAL PATIENTS | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number of strains | Number of patients | Prepatent period, av. days | Incubation period, av. days | Number of strains | Number of patients | Prepatent period, av. days | Incubation period, av. days | Number of strains | Number of patients | Prepatent period, av. days | Incubation period, av. days |
| Pacific | 18 | 86 | 13.1 | 14.4 | 3 | 3 | 15.3 | 16.0 | 18 | 89 | 13.2 | 14.4 |
| Mediterranean | 8 | 30 | 12.1 | 13.7 | 2 | 3 | 14.3 | 18.3 | 9 | 33 | 12.3 | 14.1 |
| Burma | 1 | 1 | 15.0 | 17.0 | | | | | 1 | 1 | 15.0 | 17.0 |
| Total | 27 | 117 | 12.8 ±.17* | 14.2 ±.19* | 5 | 6 | 14.8 | 17.2 | 28 | 123 | 12.9 | 14.3 |

* A test of significance applied to these means shows a difference of 5.29 standard errors which indicates that the difference between the prepatent and incubation period is real.

TABLE 2

*A Comparison of a Pacific and a Mediterranean Strain of Malaria for Prepatent and Incubation Periods*

White patients only

| STRAINS | PREPATENT PERIODS | | INCUBATION PERIODS | |
|---|---|---|---|---|
| | 1027-NG | 1031-Si | 1027-NG | 1031-Si |
| Cases | 36 | 21 | 36 | 21 |
| Means (days) | 12.8100 | 11.8571 | 13.7778 | 13.0476 |
| S.D. individual length | 1.6808 | 1.3553 | 1.8573 | 1.3619 |
| S.D. mean length | 0.2801 | 0.2985 | 0.3096 | 0.2999 |
| Standard error | 2.4991 | | 2.3585 | |

NG—New Guinea; Si—Sicily. Strain 1027-NG is also known as the "Chesson" strain.

for the comparison. The results, which are shown in table 2, indicate that infections caused by the Mediterranean strain had significantly shorter prepatent and incubation periods than did infections caused by the Pacific strain.

*Parasite-Fever Threshold.* Daily quantitative counts were made on 35 patients who had been infected by mosquitoes to determine the parasites per cmm. on the first day of fever (100 F. or over). Thirty of these patients (17 with a

Pacific and 13 with a Mediterranean strain) had a primary attack of over 9 days and were considered as having no immunity. The 17 patients with a Pacific strain (1027-NG) averaged 21 parasites per cmm. on the first day of fever; the number of parasites ranged from 3 to 60 per cmm. (fig. 1). The 13 patients with a Mediterranean strain (1031-Si) averaged 45 parasites per cmm. on the first day of fever with the number ranging from 1 to 90 per cmm. Five other patients with a Pacific strain (1027-NG) had symptoms lasting 8 days or

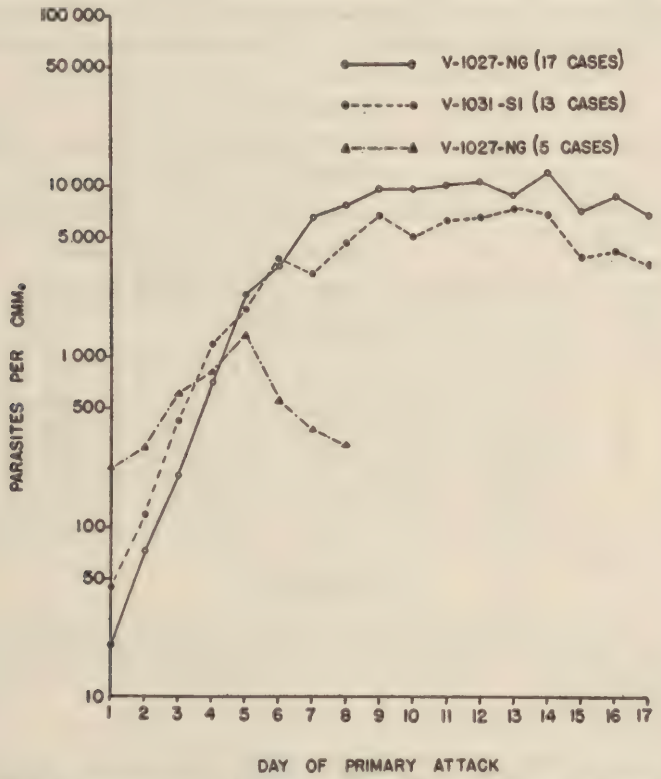



FIG. 1. RELATIONSHIP OF PARASITEMIA TO DAY OF PRIMARY ATTACK IN FOREIGN MALARIAS TRANSMITTED BY MOSQUITOES.

Five cases of v-1027-NG exhibiting a short primary attack apparently had some immunity. The other 30 cases apparently experienced a primary attack uncomplicated by immunity.

less. The average number of parasites per cmm. the first day of fever was 229, the range being from 5 to 882 per cmm. However, the parasitemia did not reach high levels, and the patients soon maintained normal temperatures. The relatively high parasite count during the first fever, the short duration of symptoms, and the relative low overall parasitemia indicate an immunity in these 5 patients.

*Relationship of Parasitemia to the Symptoms.* For the 35 patients just mentioned, the relationship of the average parasitemia to the day of primary attack

is shown in figure 1. Other data were available on the 35 patients and an additional 30, all of whom had received foreign strains of malaria by mosquito bites (table 3).

The maximum temperature is defined as the highest temperature recorded during the course of the fevers. If more than one maximum temperature occurred, the first was used in these tabulations. It is seen from table 3 that the average for the 65 cases was 106.0° F. The maximum temperature usually preceded the maximum parasitemia by several days, both tending to occur, however, in the second week of the primary attack. The maximum temperature sometimes occurred when a relatively small number of parasites were present,

TABLE 3

*Relationship of Parasitemia to Fevers in Neurosyphilitic Patients with Foreign vivax Malarias*

| STRAIN | NUMBER OF PATIENTS | FIRST MAXIMUM TEMPERATURE | | | | MAXIMUM PARASITEMIA | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | °F. | | Parasites/cmm.* | | Parasites/cmm.* | | °F. | |
| | | Range† | Average | Range | Average | Range | Average | Range† | Average |
| *Mosquito inoculated* | | | | | | | | | |
| 1005-G | 5 | 5.4–7.4 | 106.4 | 2.0–13.3 | 5.7 | 6.8–13.3 | 10.5 | 4.0–5.8 | 105.1 |
| 1019-G | 3 | 5.2–5.8 | 105.5 | 0.1– 2.8 | 1.4 | 1.5– 3.1 | 2.3 | 5.0–5.2 | 105.1 |
| 1027-NG | 34 | 4.0–7.0 | 105.9 | 0.3–44.2 | 8.1 | 1.0–44.2 | 15.6 | 2.0–6.4 | 105.0 |
| 1031-Si | 16 | 5.2–6.8 | 106.3 | 0.2–13.1 | 3.8 | 3.2–35.4 | 12.8 | 3.0–6.4 | 104.7 |
| 1032-NG | 7 | 5.8–6.2 | 106.0 | 0.5– 9.0 | 3.6 | 5.5–43.2 | 14.5 | 5.0–6.2 | 105.4 |
| Total..... | 65 | 4.0–7.4 | 106.0 | 0.1–44.2 | 6.2 | 0.8–44.2 | 13.9 | 2.0–6.4 | 105.0 |
| *Blood inoculated* | | | | | | | | | |
| 1027-NG | 10 | 5.6–6.4 | 105.7 | 1.2–42.8 | 10.2 | 6.5–55.3 | 25.3 | 4.0–6.2 | 105.4 |

* In thousands.

† Degrees above 100 F.

e.g., 100 per cmm. Parasitemias many times as great occurring later in the primary attack often failed to elicit as high a fever response.

The maximum parasitemias averaged 13,917 parasites per cmm., as compared to an average of 6,207 per cmm. which accompanied the maximum temperatures.

Thus, it is apparent that the fever response was not in direct proportion to the number of parasites present. The lesser febrile response to a higher parasite density in the latter part of the disease probably signifies a developing immunity against the effects of the parasites.

The highest parasitemias seen were 44,200 per cmm. for the Pacific strain (1027-NG) and 35,400 per cmm. for the Mediterranean strain (1031-Si).

From tables 3 and 4 and figure 1, it appears that the Mediterranean strain (1031-Si) did not produce an average parasitemia as high as the Pacific strain but that the fever response was about the same.

Of the 34 cases infected with strain 1027-NG, a comparison of the parasitemias according to whether the fevers occurred daily or every other day is as follows:

| STRAIN 1027-NG | FEVERS | |
|---|---|---|
| | Mainly quotidian (27) | Mainly tertian (7) |
| Parasites per cmm. at first maximum temperature.. | 7,100 | 12,200 |
| Parasites per cmm. at maximum parasitemia..... | 14,700 | 18,900 |

One might have expected that patients with quotidian fevers, indicating the presence of two broods of parasites, would have higher parasitemias than those with tertian fevers indicating only one brood of parasites. Such, however, was not the case.

There were data available on 10 patients who received strain 1027-NG by blood transfer, and these are also shown in table 3. Five of these received thio-

TABLE 4

*Average of Fever Peaks in 5 Strains of Induced Foreign Malarias*

| STRAIN | TOTAL PATIENTS | TOTAL PAROXYSMS | AVERAGE FEVER PEAKS |
|---|---|---|---|
| | | | °F. |
| 1005-G | 5 | 58 | 104.7 |
| 1019-G | 3 | 38 | 104.4 |
| 1027-NG | 37 | 432 | 104.4 |
| 1027-NG* | 11 | 120 | 104.7 |
| 1031-Si | 16 | 210 | 104.6 |
| 1032-NG | 7 | 76 | 104.8 |
| Average..... | 79 | 934 | 104.5 |

*Blood induced. Remainder induced by mosquito bites. NG—New Guinea; G—Guadalcanal; Si—Sicily. Strain 1027-NG also known as the "Chesson strain".

bismol early in the infection which may have influenced the time of appearance of the maximum temperatures and parasitemias. In general, the blood induced infections had higher parasitemias than the sporozoite induced infections and (although not shown in table 3) the maximum parasitemias and maximum temperatures tended to occur more nearly together than in the sporozoite induced cases. This was true whether or not the blood induced cases had received thio-bismol.

*The Height of the Fevers.* The highest temperature reading in each paroxysm above 100° F. was called the "fever peak", and these were tabulated for 934 paroxysms occurring in 79 patients. These data are shown in table 4. The fever peaks averaged 104.5° F.

The height of the fevers in relationship to duration of the primary attack is shown in figure 2 where the average fever peaks for both blood induced and mosquito induced infections are shown. The strain of malaria was 1027-NG (Chesson).

The fever peaks gradually increased during the first paroxysms. The maximum was reached usually during the second week of symptoms. This was true of all 5 strains on which data were available.

In the blood induced infections the fever peaks rose faster and maintained a higher level generally than in mosquito induced infections. As shown above, this was also true of the parasitemia in blood induced infections.

*Periodicity of Fevers.* To calculate the periodicity of the fevers, the peaks of the fever were used as reference points; the intervals between these peaks are designated as "paroxysmal intervals".

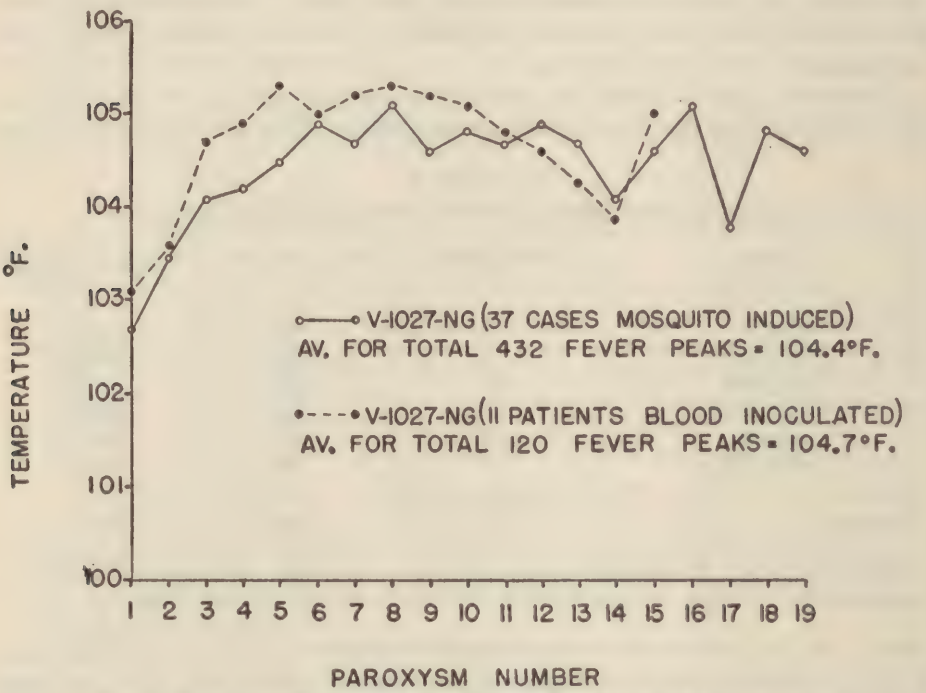



FIG. 2. THE RELATIONSHIP OF THE FEVER PEAKS TO THE NUMBER OF PAROXYSMS EXPERIENCED IN THE PRIMARY ATTACK.

The New Guinea strain (Chesson) of *P. vivax* was induced either by blood or mosquito inoculations.

As the temperature readings were taken every 4 hours and hourly when over 100° F., the peaks were evident. When the fever remained at peak for more than one reading, the first reading was used. It has been shown (8) that the fever peaks have a definite time relationship to the segmentation phases of the parasites, so that the length of time between fever peaks can be taken as the length of the asexual cycle of the parasites.

These intervals were counted only after the fevers had settled down to a tertian rhythm, either naturally or following the use of thio-bismol (8). The hours between fever peaks were measured and tabulated as whole units, being

figured to the nearest hour. The total fever intervals studied were 260, and the results are shown in table 5.

None of the strains showed a 48-hour periodicity; all were of a shorter duration. The average for all strains was 44.5 hours with the range in the 5 strains averaging between 43.6 and 45.1 hours.

TABLE 5

*Periodicities of Tertian Fevers of Foreign P. vivax Malarias*

| | PACIFIC STRAINS | | | | PACIFIC STRAINS TOTAL | MEDITERRANEAN STRAIN 1031-Si | ALL STRAINS TOTAL |
|---|---|---|---|---|---|---|---|
| | 1005-G | 1019-G | 1027-NG | 1032-NG | | | |
| No. of patients | 3 | 2 | 18 | 7 | 30 | 7 | 37 |
| No. of paroxysmal intervals | 19 | 19 | 133* | 47 | 218 | 42 | 260 |
| Mean length of paroxysmal intervals (hours) | 45.0 | 43.6 | 44.3 | 44.8 | 44.4 | 45.1 | 44.5 |
| S.D. mean (hours) | 0.35 | 0.44 | 0.15 | 0.28 | 0.12 | 0.35 | 0.12 |

* Includes 68 intervals from blood induced cases. Remainder from mosquito induced infections.

TABLE 6

*Percentage of Paroxysms Accompanied by Chills*

Arranged according to stage of disease. Only patients showing a course of more than 5 paroxysms were included.

| STRAIN NUMBER | PERCENTAGE OF PAROXYSMS ACCOMPANIED BY CHILLS | | | | TOTAL PAROXYSMS | |
|---|---|---|---|---|---|---|
| | Paroxysm number | | | | Number | Per cent with chills |
| | 1–5 | 6–10 | 11–15 | 16–20 | | |
| 1005-G | 64.2 | 96.0 | 88.9 | | 59 | 81.4 |
| 1019-G | 53.3 | 66.7 | 83.3 | | 36 | 63.9 |
| 1027-NG | 52.7 | 80.3 | 94.4 | 89.5 | 420 | 72.9 |
| 1031-Si | 58.2 | 88.5 | 100.0 | 100.0 | 144 | 79.9 |
| 1032-NG | 45.7 | 75.0 | 70.0 | | 77 | 61.0 |
| Total | 53.9 | 81.9 | 92.9 | 93.3 | 736 | 73.2 |
| Total paroxysms | 295 | 271 | 140 | 30 | 736 | |

*Relationship of Chills to Paroxysms.* The association of chills with the fevers was examined in 736 paroxysms. The patient was designated as having a chill if he complained of being cold, as well as when the overt chilling process was observed. The data are given in table 6.

From these data it is clearly shown that proportionately fewer chills accompanied the first five paroxysms than the paroxysms after the fifth.

Of the 420 paroxysms shown for 1027-NG, 325 were from blood induced

infections, and the rest were mosquito induced. Using the fourfold table, the difference between these two methods of the association of chills and fevers was shown to be 1.8 Standard Errors which is not taken to be significant. Of the total 736 paroxysms, 416 were from blood induced infections. The difference between blood and mosquito induced methods for the total number was also insignificant (0.1 Standard Error).

As the maximum parasitemias usually occurred after the fifth paroxysm, this suggests that the rigors might have some relationship to the number of parasites present.

*Type of Fever at Onset.* The type of fever at onset in 58 cases induced by mosquito bites follows:

| SEQUENCE OF FEVERS | NO. OF CASES | TOTAL | PER CENT OF TOTAL CASES |
|---|---|---|---|
| Remittent followed by: | | 24 | 42 |
| quotidian solely | 14 | | |
| tertian solely | 6 | | |
| quotidian, then tertian | 3 | | |
| tertian, then quotidian | 1 | | |
| Quotidian followed by: | | 29 | 50 |
| quotidian solely | 18 | | |
| tertian solely | 9 | | |
| tertian, then quotidian | 2 | | |
| Tertian followed by: | | 5 | 8 |
| tertian solely | 4 | | |
| quotidian solely | 1 | | |

One-half of the cases started as quotidian, 42 per cent as remittent, and only 8 per cent as tertian. Of those changing eventually to tertian, regardless of onset type, there were 22 (38 per cent) and those changing to quotidian eventually were 36 (62 per cent). Most of those which started either as quotidian or tertian continued as such. Only a few starting as remittent fever remained so long enough (3 to 4 days) to necessitate using thio-bismol (sodium bismuth thioglycollate) to convert them to tertian periodicity. These were not included in the above table.

Of 8 blood inoculated cases studied, none started as remittent, 2 started and remained tertian, 2 started quotidian and changed to tertian, one showed a quotidian-tertian-quotidian development, 2 started and remained quotidian, and one started tertian and changed to quotidian.

The 24 remittent fevers were distributed according to duration as follows: 2 days, 9; 3 days, 9; 4 days, 6.

*Use of Sodium Bismuth Thioglycollate to Convert Paroxysms to Tertian Periodicity.* Sodium bismuth thioglycollate (thio-bismol) was given when one brood of parasites was half-grown to convert remittent fevers to a tertian periodicity. It was given also to 12 cases of quotidian paroxysms, and 11 were changed to

tertian occurrence. The one failure may have been due to giving the drug at the wrong time. The malarias involved were 2 strains from the Pacific (1005-G and 1027-NG) and one from the Mediterranean (1031-Si). Five infections originated from blood transfer, and the remainder were induced by mosquito bites. Thus, the use of sodium bismuth thioglycollate to regulate the paroxysms of foreign *vivax* malaria seems to be quite dependable. A similar effect is observed when the drug is administered to patients infected with the St. Elizabeth strain of *P. vivax* (10).

*Length of Primary Attack.* Data on the number of paroxysms in 60 patients infected by mosquito bite were available and are shown in table 7.

Only 12 (20 per cent) cases terminated spontaneously. These averaged 8.5 paroxysms with the range extending from 4 to 14 paroxysms. The remainder (80 per cent) were treated to terminate the infections, some of which had shown 22 paroxysms.

TABLE 7

*Number of Paroxysms in the Primary Attack (Mosquito Induced)*

| STRAIN | TERMINATION | | | | |
|---|---|---|---|---|---|
| | SPONTANEOUSLY | | | BY TREATMENT | |
| | Number patients | Av. no. paroxysms | Range | Number patients | No. paroxysms range |
| 1005-G | 0 | 0 | 0 | 5 | 11–14 |
| 1019-G | 0 | 0 | 0 | 3 | 11–13 |
| 1027-NG | 8 | 7.5 | 4–14 | 26 | 9–20 |
| 1032-NG | 3 | 9.7 | 8–11 | 4 | 11–14 |
| 1031-Si | 1 | 13 | 13 | 10 | 8–22 |
| Totals and averages..... | 12 | 8.5 | 4–14 | 48 | 8–22 |

Of the 10 patients inoculated by blood with strain 1027-NG (which are not shown in table 7), only 2 (20 per cent) cases were self-terminated; one after 6 and one after 11 paroxysms respectively. Of those terminated by treatment, some had shown up to 22 fevers.

*Heterologous Strain Immunity.* Nine patients were given both native and foreign strains of *P. vivax* to determine whether heterologous immunity developed. These data are detailed in table 8.

Eight cases were first given the St. Elizabeth strain of *P. vivax*. The termination of the primary attack was spontaneous in these cases. In some cases parasitemias persisted after the last paroxysm, of which some were cleared by treatment before reinoculation. From 16 to 44 days after the last paroxysm of the primary attack, patients were inoculated with a foreign strain. All developed parasitemias and paroxysms of varying lengths, some of which (AMR, NB, JMD, and LG) approximated normal primary infections.

The ninth case (RSJ) was given first a foreign malaria which, after spontaneous termination, was followed by inoculation with the St. Elizabeth strain. The latter strain also produced a symptomatic infection consisting of 8 paroxysms.

The parasitemia at the first paroxysm of the second inoculation averaged higher than at the first paroxysm of the first inoculation. This might indicate some immunity following the first infection, particularly in those cases where the second parasitemias were many times higher (NB, LG, and AC). However, this pattern was not consistent as some of the fever threshold parasitemias of the second infection (OCJ and JBH) were lower than those of the first infection.

It is obvious, therefore, that the first infection did not produce enough immunity to prevent a different strain from developing subsequently. Furthermore, if any immunity was developed, it was not very effective in some cases from a parasitological viewpoint. This is similar to the results obtained by

TABLE 8

*Heterologous Immunity. Foreign vs. Native Strains of P. vivax*

| PATIENTS | FIRST INOCULATION | | | | | | SECOND INOCULATION | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Strain | Method | No. parox. | Terminated | Parasites per cmm. | | Day from last primary paroxysm | Strain | Method | No. parox. | Terminated | Parasites per cmm. | |
| | | | | | First parox. | Last praox. | | | | | | First parox. | Last parox. |
| O. C. J..... | SE | M | 6 | S | 1,708 | 308 | 16 | 1512-NG | M | 7 | S | 62 | 712 |
| J. M. D.... | SE | M | 2 | S | 6,750 | 5,850 | 42 | 78-NG | M | 20 | T | 7,550 | |
| N. B..... | SE | B | 16 | S | 110 | 1,930 | 35 | 1512-NG | M | 10 | T | 10,650 | |
| A. C..... | SE | B | 9 | S | 0 | 1,570 | 18 | 90-NG | M | 7 | S | 3,150 | 140 |
| L. G..... | SE | B | 13 | S | 360 | 550 | 18 | 94-NG | M | 11 | T | 10,350 | |
| A. M. R.... | SE | B | 8 | S | 180 | 7,118 | 44 | 1512-NG | B | 11 | S | 1,250 | 2,212 |
| J. B. H.... | SE | B | 21 | S | 638 | 1,275 | 31 | 109-B | B | 3 | S | 625 | 3,362 |
| B. H..... | SE | M | 17 | S | 725 | 7,075 | 31 | 1512-NG | B | 5 | S | 11,750 | 9,250 |
| R. S. J..... | 109-B | B | 12 | S | 75 | 1,312 | 38 | SE | B | 8 | S | 1,950 | 625 |
| Total average..... | | | 11.6 | | 1,172 | 2,999 | | | | 9.1 | | 5,260 | |
| Average for 6 whose second infection was spontaneously terminated..... | | | 12.2 | | 554 | 3,110 | | | | 6.8 | | 3,131 | 2,717 |

S—spontaneously; T—by treatment; M—transmitted by mosquitoes; B—transmitted by blood; SE—St. Elizabeth strain; 109-B—Burma strain; NG—New Guinea strain.

Kaplan *et al.* (5), who, using American and Pacific strains, found that "the reinfection of a previously *vivax*-infected patient with a heterologous strain would, on the average, produce clinical paroxysms totaling approximately 75 per cent of the clinical paroxysms experienced on original infection".

## DISCUSSION

*Periodicity.* Of the strains studied in detail, regardless of origin, none showed a 48-hour interval between fever peaks but, rather, a shorter periodicity. This is true of the five strains detailed in table 4, as well as a New Hebrides strain

reported earlier (8). Two American strains studied earlier also showed a shorter periodicity (8).

Kitchen (6) found the periodicity of the McCoy strain of *P. vivax* to be only 16 minutes short of 48 hours, whereas the periodicity of the strains, both native and foreign, studied by us averaged from 43.6 to 45.1 hours in length. However, there are points of difference in our observations. Apparently, most of Kitchen's observations were made on infections characterized by quotidian fevers; ours were made only upon fevers appearing every other day, either naturally or after conversion by administration of sodium bismuth thioglycollate. Furthermore, he found that the shorter cycles characterized rigorless paroxysms primarily and prevailed consistently only during the first week of the attack. As we waited for the conversion to the tertian periodicity, most of our readings were made during and subsequent to the second week of fever. As shown in table 6, chills accompanied the fevers less frequently during the first 5 fevers (about the first week) than after this time.

The difference in periodicities may be due to the fact that Kitchen measured quotidian fevers primarily, and we measured the tertian type only. It has been obvious in our work, even without measuring, that the tertian type of fever does not recur at 48-hour intervals, but shows a shorter periodicity.

*The Use of Foreign Malarias as a Therapeutic Agent against Neurosyphilis.* The foreign malarias tested appeared from a parasitological viewpoint to be satisfactory as a therapeutic agent in the treatment of white neurosyphilitic patients. Most of the white patients (95 per cent) became infected (9) after being bitten by infected mosquitoes. The prepatent and incubation periods were relatively short (table 1). Most of the infections (80 per cent) produced a satisfactory number of paroxysms (10 to 20). The infections which were self-terminated averaged 8.5 paroxysms, about the number of paroxysms desired by some clinicians. The parasite count seldom reached densities high enough to require drug intervention. The use of sodium bismuth thioglycollate was shown to be reliable in reducing the quotidian fevers to a tertian periodicity. Previous infection with native malarias seemed to produce little immunity against subsequent infections with these foreign strains. Other workers (4) using these same strains have shown that the infections responded promptly to adequate treatment.

But, as shown previously (9), these *vivax* malarias were not satisfactory in the treatment of Negro neurosyphilitics as most of these patients did not develop the infection.

## SUMMARY AND CONCLUSIONS

1. White neurosyphilitic patients were infected with foreign *Plasmodium vivax* malaria, both by blood inoculation and by mosquito bite. Infections resulting from mosquito transmission showed the averaged prepatent period to be 12.8 days and the average incubation period to be 14.2 days. These periods were significantly shorter for the Mediterranean strains than for strains from the Pacific.

2. In the mosquito transmitted infections, the first maximum fever usually preceded the maximum parasitemia by several days. The average of the first maximum fever was 106.6° F. The maximum parasitemia averaged 13,900 parasites per cmm.

3. In the blood transmitted infections, the first maximum fevers and the maximum parasitemias usually occurred at about the same time. The maximum parasitemias were higher than in mosquito induced infections.

4. The tertian type paroxysms showed an average periodicity of 44.5 hours, ranging from 43.6 to 45.1 hours. None showed a 48-hour periodicity.

5. Chills accompanied the fevers in 73.2 per cent of the cases. Chills were less frequently present with the first 5 fevers than with the later fevers.

6. The types of fever at onset of mosquito induced infections were: quotidian, 50 per cent; remittent, 42 per cent; and tertian, 8 per cent. These types were often succeeded by a different type.

7. Sodium bismuth thioglycollate was reliable in changing remittent and quotidian paroxysms to tertian occurrence.

8. Usually, the primary infections produced over 10 paroxysms.

9. Little or no heterologous immunity was demonstrated between 5 foreign strains and the St. Elizabeth strain of *P. vivax*.

10. The foreign malarias appear to be satisfactory as a therapeutic agent to treat white neurosyphilitic patients. This was not true of Negro patients.

## REFERENCES

1. Boyd, M. F., and Stratman-Thomas, W. K. 1933 Studies on benign tertian malaria. 4. On the refractoriness of negroes to inoculation with *Plasmodium vivax*. Am. Jour. Hyg., **18**(2): 485–89.
2. Earle, W. C., and Perez, M. 1932 Enumeration of parasites in the blood of malarial patients. Jour. Lab. & Clin. Med., **17**: 1124–.
3. Ehrman, F. C., Ellis, J. M., and Young, M. D. 1945 *Plasmodium vivax* Chesson strain. Science, 101(2624): 377.
4. Gordon, H. H., Marble, A., Lippincott, S. W., Hesselbrock, W. B., and Ellerbrook, L. D. 1946 Clinical and laboratory studies of relapsing *vivax* malaria of Pacific origin. New Eng. Jour. Med., **234**: 519–23.
5. Kaplan, L. I., Becker, F. T., and Boyd, M. F. 1946 Homologous and heterologous strains of *Plasmodium vivax:* a cross-inoculation study of malaria strain immunity. Jour. Lab. Clin. Med., **31**: 400–408.
6. Kitchen, S. F. 1946 Observations on the character of the paroxysms in *vivax* malaria. Jour. Nat. Mal. Soc., **5**(1): 57–78.
7. Moore, J. A., Young, M. D., Hardman, N. F., and Stubbs, T. H. 1945 Studies on imported malarias. 2. The ability of California anophelines to transmit malarias of foreign origin and other considerations. Jour. Nat. Mal. Soc., **4**: 309–329.
8. Young, M. D. 1944 Studies on the periodicity of *Plasmodium vivax*. Jour. Nat. Mal. Soc., **3**: 237–40.
9. Young, M. D., Ellis, J. M., and Stubbs, T. H. 1946 Studies on imported malarias. 5. Transmission of foreign *Plasmodium vivax* by *Anopheles quadrimaculatus*. Am. Jour. Trop. Med., **26**(4): 477–82.
10. Young, M. D., McLendon, S. B., and Smare, R. G. 1943 The selective action of thio-bismol on induced malaria. Jour. Am. Med. Assn., **122**: 492–94.
11. Young, M. D., Stubbs, T. H., Ellis, J. M., Burgess, R. W., and Eyles, D. E. 1946 Studies on imported malarias. 4. The infectivity of malarias of foreign origin to anophelines of the southern United States. Am. Jour. Hyg., **43**: 326–41.

Reprinted from THE JOURNAL OF THE NATIONAL MALARIA SOCIETY
Vol. 7, No. 1, March, 1948

# STUDIES ON IMPORTED MALARIAS

## 7. THE PARASITOLOGICAL PATTERN OF RELAPSING *Plasmodium vivax* IN MILITARY PATIENTS[1]

DON E. EYLES AND MARTIN D. YOUNG[2]

*Office of Malaria Investigations, National Institute of Health, Milledgeville, Ga., and Columbia, S. C.*

(Received for publication 5 May 1947)

Earlier reports in this series (Young, *et al.*, 1945, 1946a and b; Moore, *et al.*, 1945) have demonstrated the ability of the principal malaria vectors of this country to transmit foreign malarias from returned military personnel showing clinical relapses. Upon determining that clinical relapsing cases readily infected the mosquito vectors, it was desirable to determine if the human carriers of malaria could infect mosquitoes at times other than during clinical relapses, viz., during asymptomatic parasitic relapses (when parasites were present in the blood stream without clinical symptoms).

During the study of this problem it became apparent that the relapsing parasites were found in several patterns in relation to the clinical manifestations of the disease. The delineation of these parasitic patterns by the study of a large group of patients is the object of this report.

The present study was conducted at Moore General Hospital and extended from October, 1944 until March, 1946. During the 18 months, over 700 patients with *vivax* malaria were studied in whom over 1,000 individual clinical attacks were observed. Of these, more than 200 were "delayed" primary attacks, occurring after termination of atabrine suppression. The size and nature of the study can best be demonstrated by tabulation (Table 1).

### METHODS

Patients suspected of having malaria were admitted to the malaria wards. After diagnosis was proved by the finding of parasites in the blood smears, quantitative parasite determinations were made daily or more often until three consecutive negative smears were obtained, this usually following treatment. Counts referred to in this study as fever threshold counts are the first quantitations after the patients were admitted to the ward. These usually followed the first paroxysm by less than 12 hours.

After the treatment of clinical attacks, most patients were transferred to convalescent wards and were followed with twice-weekly smears until relapse or until discharge to duty (usually after 120 or more days of observation without clinical at-

[1] Contribution from the Imported Malaria Studies program of Malaria Investigations, National Institute of Health and the Office of Malaria Control in War Areas, United States Public Health Service, Columbia, S. C.

[2] Moore General Hospital made available the relapsing cases, as well as the laboratory quarters, for which we express our appreciation. We are also indebted to the Office of The Surgeon General, United States Army, whose active interest made the program possible.

tack). Personnel limitations necessitated the study of some patients only during their clinical attack. Asymptomatic patients found to have parasites during the twice-weekly follow-up were not transferred to malaria wards, but were required to report for temperature readings three times daily and were examined for parasites daily.

To determine parasite density, a minimum of 0.1 cmm. of blood was examined unless parasite density was very high. During the initial phases of the study quantitations were made by determining the ratio of parasites to leucocytes. This method was abandoned early in the study for a modification of the direct method described by Earle and Perez (1933).

Patients were considered symptomatic only when oral temperatures exceeded 100°F.

TABLE 1

*Scope of study of foreign* Plasmodium vivax *in military personnel*

| THEATER | TOTAL NUMBER OF PATIENTS | NUMBER OF OBSERVED CONSECUTIVE ATTACKS WITH NUMBER OF PATIENTS UNDER EACH CATEGORY | | | | | | TOTAL NUMBER OF OBSERVED ATTACKS |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | |
| Pacific | 563 | 361 | 126 | 56 | 14 | 4 | 2 | 869 |
| Mediterranean | 155 | 125 | 25 | 5 | — | — | — | 190 |
| CBI | 11 | 8 | 1 | 2 | — | — | — | 16 |
| Caribbean | 2 | 2 | — | — | — | — | — | 2 |
| Total | 731 | 496 | 152 | 63 | 14 | 4 | 2 | 1,077 |

## OBSERVATIONS

*Clinical relapse attacks.* Over 800 clinical relapses were observed in this study. These relapses occurred both in patients whose primary attacks (delayed) had been observed at this hospital and in patients returning from overseas with malaria histories.

The first warning of an approaching clinical episode in those patients under observation before the onset of the first paroxysm was often the presence in the blood of small numbers of malaria parasites. In our study 77.2 per cent of the relapse attacks in patients under observation (351 cases) were first detected by the finding of parasites in the blood smears before the first fever occurred. Undoubtedly this percentage would have been increased had smears been made on a daily rather than twice-weekly basis, this being logical because of high fever threshold values at relapse. These parasitemias are henceforth termed "preclinical asymptomatic parasitemias."

On the average the first parasites were found 3.5 days before the first paroxysm of the relapse. Ordinarily, there was a steady increase in parasite number until the fever threshold level was reached. In a few instances extended asymptomatic parasitemias culminating in clinical relapse occurred; some upset of the immune balance must have allowed the parasites to increase to symptom-producing level after having been held in check for days.

The median fever threshold parasite count for over 800 relapse attacks was 3200

per cmm. (mean, 6300 per cmm.).[3] The median for Pacific cases was 2952 per cmm. (mean, 6030), for Mediterranean cases was 3836 (mean, 7250). Table 2 summarizes the counts at symptomatic relapse by theater and by relapse number, grouping the attacks in order to increase the number of patients in each category. No significant variation in parasite level was found between early, middle, and late relapses in either the Pacific or the Mediterranean group. The Mediterranean relapse cases showed significantly higher parasite fever threshold counts than the Pacific group ($p = 0.017$).

To further test the conclusion above that no significant change occurred from early to late relapses, fever threshold counts on successive relapses were tabulated for 265 cases. Counts at first observed relapse had a median value of about 4000 per cmm.

TABLE 2

*Median and mean parasite counts at symptomatic relapse grouped according to number of relapses patients had undergone. The grouping has the effect of increasing the number of patients in the various categories*

| THEATER | NUMBER OF RELAPSES | | | |
|---|---|---|---|---|
| | 1–5 | 6–10 | 11 and over* | All |
| Pacific | | | | |
| Median count‡ | 2752 | 3182 | 2808 | 2952† |
| Mean count‡ | 5850 | 7150 | 5630 | 6035 |
| Number cases | 446 | 108 | 105 | 659 |
| Mediterranean | | | | |
| Median count‡ | 3516 | 3790 | 4320 | 3836† |
| Mean count‡ | 6830 | 7330 | 7428 | 7249 |
| Number cases | 40 | 90 | 55 | 185 |

* Few patients reported over 20 relapses.

† The difference ($p = 0.017$) between these values is considered as significant.

‡ Parasites per cmm.

Counts at second observed relapse had a median value of 3900. The difference is insignificant.

The same tabulation of consecutive attacks for the same patients does demonstrate a factor of importance; a significant although moderate degree of positive correlation between counts during successive episodes was noted ($r = +.4$). Thus, patients with high counts during one episode were likely to have high counts at a second and vice versa.

Routine counts made on 844 relapse attacks showed gametocytes to be present in 35.2 per cent. The above percentage refers to male gametocytes since only this form is easily recognized in the thick film which was used for most of the counts. Table 3 summarizes the gametocyte counts by theater and by relapse number.

Pacific cases showed gametocytes in only 29.4 per cent of 659 attacks against 55.7 per cent for 185 Mediterranean attacks. Analysis shows this difference of 26.3 per

[3] Fever threshold counts are not normally distributed, consequently both median and mean counts are given; in our opinion the median is the best representation of central tendency.

cent to be highly significant. Median gametocyte count for Mediterranean cases was also higher being 110 per cmm. compared with 80 per cmm. for the Pacific group.

The apparent lower gametocyte counts in later relapses for the Pacific cases was not significant. In the group of Mediterranean cases the later attacks had significantly lower gametocyte counts ($p = .011$ when Mediterranean relapses 1 through 5 were compared with relapses 11 and over).

In a series of 265 patients studied during consecutive attacks no significant difference in gametocyte incidence was noted from the first observed to the second observed relapse attack. In this series 56 patients showed gametocytes during both attacks, 139 during neither attack, and 70 showed sexual forms during one attack and not during the other. Thus 195 of the 265 patients were consistent which would make it appear that some patients are more likely to produce gametocytes than others and therefore more likely to be efficient infectors of mosquitoes. This is confirmed by the observation that of 90 patients found with gametocytes during the first clinical

TABLE 3

*Proportion of clinical attacks during the course of which male gametocytes were noted. Grouped according to theater and the number of relapses the patients had undergone*

| THEATER | DELAYED PRIMARY ATTACK | NUMBER OF CLINICAL RELAPSES | | | |
|---|---|---|---|---|---|
| | | 1–5 | 6–10 | 11 and over | Total relapses |
| Pacific | | | | | |
| Number cases | 200 | 446 | 108 | 105 | 659 |
| Per cent with gametocytes | 22.5 | 29.6 | 36.1 | 21.9 | 29.4 |
| Mediterranean | | | | | |
| Number cases | | 40 | 90 | 55 | 185 |
| Per cent with gametocytes | | 75 | 56 | 49 | 55.7 |

attack, 56 or 62.2 per cent had gametocytes during the second (as opposed to 34 per cent of original sample with gametocytes). This higher incidence would rarely result from chance (p below 0.00006).

To determine if there was any relationship between the height of parasitemia at onset of symptoms and the likelihood of subsequent relapse, data on 200 Pacifi theater patients who had been observed after relapse for 120 days or until relapse again occurred were tabulated. Table 4 shows that similar proportions relapsed in the groups with different parasite levels. A gradual increase in the proportion relapsing was noted as parasite level increased but none of these values differed significantly from any other.

*Delayed primary attacks.* Over 200 patients were observed through the course of a clinical attack reported by them to be their first. These attacks in individuals without malaria history are termed "delayed" primaries since exposure to infected mosquitoes obviously occurred when under suppressive atabrine discipline (or perhaps rarely quinine). In many instances the history of the patient indicated a definite locality of origin for the infection but often the patient had been in several malarious areas and only a guess could be hazarded. With very few exceptions, the

delayed primaries studied were from the Pacific theater of operations. The Territory of New Guinea probably contributed the greatest number of infections.

Delayed primary attacks occurred on the average 49.1 days after discontinuation of suppressive atabrine. One hundred cases analyzed gave a median value of 41 days, the central half occurring between 27 and 58 days. Extreme intervals between discontinuation and onset were 7 and 167 days. The persistence of a therapeutic atabrine level in the blood doubtless explains the delay until several weeks. The variation in interval after discontinuation of atabrine may be due to the relationship of the time of drug discontinuation to the not yet fully explained but apparently cyclic invasion of parasites to the blood from the exoerythrocytic hiding places.

The median value of the first parasite count after onset of fever for the delayed primary group was low being 870 per cmm. for 197 cases (mean 3900 per cmm.). We suspect that in this group are included some patients who had had previous unrecognized primaries because we found a group of 50 naturally induced Pacific primaries had a median fever threshold count of less than 100 parasites per cmm. Under

TABLE 4

*Relationship between parasite level at onset of symptoms and proportion of patients subsequently relapsing (Pacific theater only)*

| PARASITE LEVEL AT RELAPSE | NUMBER OF PATIENTS | RELAPSED SUBSEQUENTLY | |
|---|---|---|---|
| | | Number | Per cent |
| Parasite count below 1000 per cmm. | 34 | 24 | 71 |
| Parasite count between 1000 and 2952 per cmm. | 53 | 41 | 77 |
| Parasite count between 2952 and 5000 per cmm. | 37 | 30 | 81 |
| Parasite count over 5000 per cmm. | 76 | 63 | 83 |

combat conditions oftentimes vague illnesses did occur and frequently hospital facilities were not available for accurate diagnosis. Furthermore, atabrine discipline was likely to be most lax and bodily resistance least during the exposure necessitated by a campaign.

In the case of the relapse attacks discussed in a previous section, we believe that the first parasite count after onset of symptoms gives a fair representation of the fever threshold count. This is probably not true of the counts on the delayed primary group. On some occasions initial symptoms occurred during the course of the patient's "overseas" furlough, these symptoms persisting undiagnosed until the return of the patient to the hospital. Unfortunately for study, many of the patients were on furlough four to eight weeks after return from abroad for Army policy was to allow these returning patients to take leave as soon as possible after return to the United States.

Even though the patients with delayed primary attacks were present in the hospital at the time of onset of symptoms, delay in reporting them often occurred. Most of the patients were on dermatological, surgical, or gastro-enterological wards and, having had no previous experience with the disease, did not report their malaise promptly.

To eliminate this factor of delay, 63 cases, in which it was fairly certain symptoms were reported promptly, were selected and the median value of this group was 450 parasites per cmm. This is significantly lower than the median value of 2952 per cmm. for Pacific relapse attacks. For contrast 17 delayed primary cases, in which parasite counts did not follow promptly the onset of symptoms, had a median value of 1856 per cmm.

A group of 65 patients was observed during the delayed primary attack and during a subsequent relapse. Of these 44 had a higher parasite count at relapse despite the fact that these patients were carefully watched for reappearance of symptoms. Chi-square test shows this to be significantly different from the expected ratio if delayed primary attack and first relapse were similar. Median value for the 65 delayed primaries was 730 parasites per cmm.; median counts for the corresponding 65 relapses was 1980 per cmm.

The entire group of delayed primaries had gametocytes in 22.5 per cent of the attacks. This percentage was reduced to 17.5 per cent for the group in which it was certain counts followed soon after the onset of symptoms. This percentage is considerably higher than the percentage found in the naturally induced group referred to above just after onset of symptoms (2.0 per cent). It seems probable that the delay in reporting and the possible admixture of relapse attacks in the delayed primary group may be responsible for the relatively high incidence of gametocytes just as it seemed responsible for the higher total parasite counts. The values for the group of delayed primaries were lower than the incidence for Pacific relapses but the difference was only of borderline significance (p = about .05).

*Interval between clinical episodes.* The mean interval between clinical episodes in 292 instances was 61.1 days. All patients were observed for 120 or more days after each clinical attack unless relapse occurred and the cumulative frequency curve to time of relapse (Figure 1) would indicate that most of the relapses had occurred by the end of this period. Consequently, the true mean would be somewhat but not greatly larger than the figure given above, due to the non-inclusion of the few attacks with extremely long interval periods. Median value for time to relapse was near the mean, being 59 days. Length of interval as used by us is measured from the onset of one attack to the onset of the next.

The interval between relapses varied according to the drug regimen used in therapy, being shortest for quinine and longest for chloroquine. As was brought out by Most *et al.* (1946), who studied the same group of patients, this variation in interval is likely related to the degree of persistence of a therapeutic drug level in the tissues of the body.

After treatment of the clinical attacks, low level, asymptomatic, usually transient parasitemias were noted in a small proportion of the cases; this type of parasitemia is henceforth termed in this paper as "interval asymptomatic parasitemia". Two hundred and sixty-two intervals were studied with twice-weekly smears and 12.2 per cent showed this phenomenon (Table 5). In most cases temperatures were taken on patients that showed such parasites to determine if sub-clinical response to the infection was occurring and in the instances studied no such sub-clinical response was noted.

Proportions showing the interval parasitemia were so nearly similar for the two major theaters that these are not discussed separately. The parasitemias were on

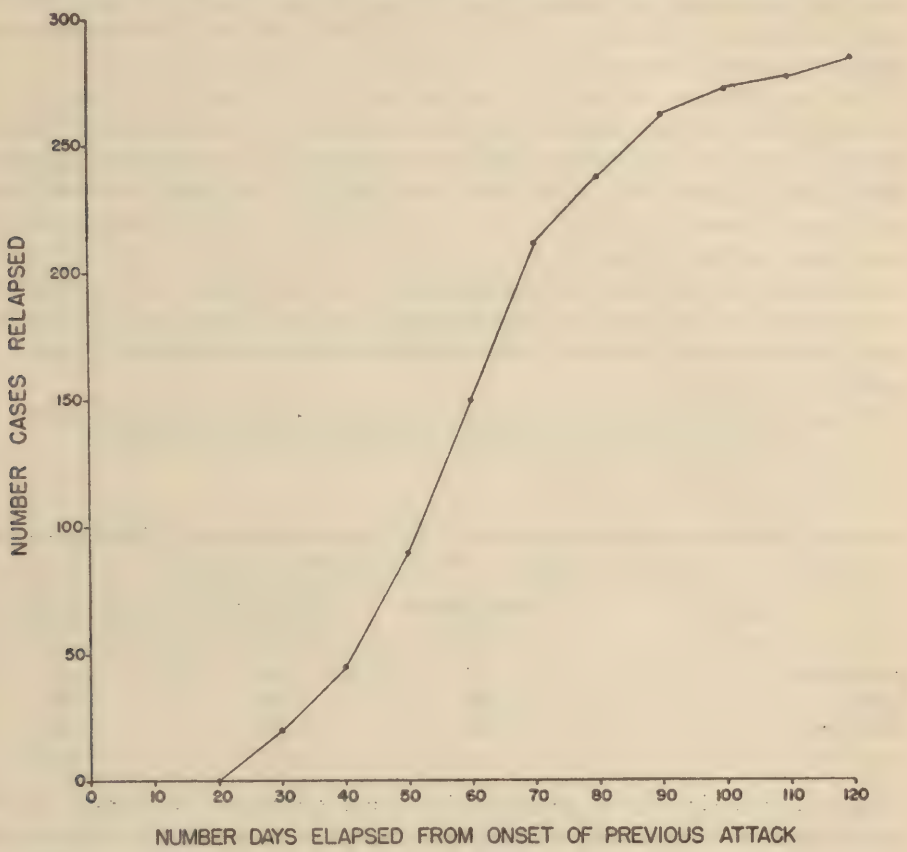



FIG. 1. Cumulative frequency graph of interval to relapse. Interval in these cases is measured from the onset of the nth attack to the onset of the nth + 1 attack.

TABLE 5

*Incidence of interval asymptomatic parasitemias in 263 patients. Blood smears taken twice weekly*

| THEATER | NUMBER INTERVALS OBSERVED | NUMBER WITH INTERVAL PARASITEMIA | PER CENT WITH INTERVAL PARASITEMIA |
|---|---|---|---|
| Mediterranean | 32 | 3 | 9.4 |
| Pacific | 231 | 29 | 12.6 |
| Total | 263 | 32 | 12.2 |

the average first noted 56.1 days after the onset of the preceding clinical episode. This corresponds so closely to the mean time to relapse that the coincidence suggests that these parasitemias are homologous to clinical relapses, the parasites being held in check and finally eliminated by the immune mechanism of the human host.

The interval asymptomatic parasitemia endured on the average for 12 days but the cases actually observed varried from one to 62 days. In over two-thirds of the cases the patient was continuously positive until the negative period preceding relapse. The mean time from the end of the interval parasitemia to the following clinical relapse was 23.8 days.

Thus, from the onset of the interval asymptomatic parasitemia to the onset of the following clinical episode was on the average 35.8 days. This might lead one to believe that a further invasion of the blood from the hypothetical exoerythrocytic situations occurred at this time. The 35-day time interval is not greatly different from the interval between attacks when the first attack is terminated by a quickly eliminated drug such as quinine.

Parasite level during the interval parasitemia was lower than that found in the same patient at the following clinical relapse. Median highest level during the asymptomatic period was about 270 as compared with a value near 3000 for the following clinical episode.

The occurrence of gametocytes during the interval asymptomatic parasitemia is discussed in an accompanying paper (Eyles, Young, and Burgess 1948).

TABLE 6

*Incidence of terminal asymptomatic parasitemias in 314 patients observed over a 120-day period after last clinical expression. Blood smears taken twice weekly*

| THEATER | NUMBER PATIENTS OBSERVED | NUMBER WITH PARASITEMIA | PER CENT WITH PARASITEMIA |
|---|---|---|---|
| Mediterranean | 95 | 24 | 25.3 |
| Pacific | 219 | 55 | 25.1 |
| Both Theaters | 314 | 79 | 25.2 |

*Observations during 120 day period after last clinical expression of the disease.* Three hundred and fourteen patients were followed with twice-weekly blood smears for 120 or more days after their last observed clinical episode (usually 120 days plus length of treatment period which varied with drug). Referring again to the cumulative frequency graph (Figure 1), it will be seen that the curve becomes almost asymptotic after 120 days. Though a few relapses occur after this length of time, the number is sufficiently small that the data on a group of several hundred patients should indicate expectations during the final stages of the disease.

Our data indicate that the course of the disease in these patients usually ended with the treating of a clinical attack. However, a significant proportion, 25 per cent, were shown to have asymptomatic parasite activity after the last clinical episode, what we shall designate as "terminal asymptomatic parasitemias" (Table 6). Since proportions were similar from the two theaters, they are discussed together.

Terminal asymptomatic parasitemias were first observed on the average about 80 days after the last observed clinical attack (compare a period of about 60 days to relapse). Median day to parasite occurrence (77.5 days) was close to the mean. The mean duration of these parasitemias was about 44 days but this figure is deceptive because of the great range (1 to 186 days) and the fact that many of the parasitemias

had not terminated at the end of the observation period (nearly 35 per cent were positive less than two weeks before the end of observation).

Duration of the parasitemia as used in this text refers to the number of days elapsing between the first and last positive smear. Nearly half of them persisted for more than a month. Parasitemias were both remittent and intermittent; only those of short duration were continuously positive.

The level of parasitemia was variable. In general the maximum levels occurred during the first days of the terminal parasitemia. Occasionally a second or third lower peak was attained and in the long duration parasitemias which were nearly continuous the parasitemia was remittent but gradually decreasing in magnitude. Levels attained by the asymptomatic parasitemias were usually only one-fourth to one-half the levels noted in the same patients at the time of previous clinical attack.

Patients who showed these asymptomatic terminal parasitemias were required to report for temperature measurements three times daily. A lag in initiating tem-

TABLE 7

*Prevalence of parasitemia in 200 patients observed an average of 4 months each*

| | PACIFIC THEATER | MEDITERRANEAN THEATER | BOTH THEATERS |
|---|---|---|---|
| Number patients observed | 150 | 50 | 200 |
| Number attacks | 232 | 61 | 293 |
| Mean number of attacks per patient | 1.6 | 1.2 | 1.5 |
| Mean days with parasitemia | 16.8 | 12.9 | 15.8 |
| Mean days with parasitemia accompanied by symptoms | 3.9 | 2.8 | 3.6 |
| Mean days with asymptomatic parasitemia | 12.9 | 10.1 | 12.2 |
| Preclinical | 5.7 | 4.5 | 5.4 |
| Interval | 1.5 | 0.8 | 1.3 |
| Terminal | 5.8 | 4.8 | 5.5 |

perature determinations of from one to two days often followed the first appearance of parasites and there is some possibility that minimal clinical symptoms occurred, but these, if present, were in no case of sufficient severity that the patient reported for sick call. Several of the parasitemias started while patients were on furlough; consequently, no temperature record was available. These patients reported no attack and brought back smears taken twice-weekly while away from the post.

In two instances patients placed in the category of those with terminal asymptomatic parasitemias were detected with slight fevers. In both of these cases levels of parasitemia similar to those attained in the previous clinical attack were reached for a short time. In both cases temperatures did not rise as high as 102°F. and fell immediately without treatment.

*Relative prevalence of parasitemia.* To determine the proportion of time that malaria patients exhibited parasitemias, a group of 200 patients were followed for an average of slightly over four months with blood smears taken no less frequently than twice weekly. The data thus secured are shown in Table 7.

During the four-month period, parasites were present in the peripheral blood of Pacific theater patients on the average for 16.8 days or 13.0 per cent of the time.

During most of this time (12.9 days) the patients were asymptomatic; this time of parasitemia without symptoms amounting to 10.0 per cent of the four-month observation period. On the average both the preclinical and the terminal asymptomatic parasitemias persisted longer than the clinical parasitemia. It should be pointed out that patients were usually treated within 24 to 48 hours of the first symptoms.

Parasites were present in the blood of Mediterranean patients on the average for 12.9 days or 10 per cent of the four-month period. The fact that Pacific cases relapse more frequently than Mediterranean accounts for some of the difference since the Pacific cases were observed to average 1.6 attacks each in the four-month period contrasted to an average of 1.2 attacks for the Mediterraneans. Chance probably is responsible for the small differences in prevalence of asymptomatic parasitemia.

*Observations of untreated attacks.* A small group of patients volunteered to deny themselves of treatment; this was suggested with the view that by suffering longer, immunity might reach a height sufficient to prevent further relapse.

Of ten patients originally in this group, three were treated because of the severity of the continued attack. One of the seven patients that succeeded in going through with the clinical phase of the attack without treatment was treated at his own request several months later although he was asymptomatic at that time. All seven of the patients had terminal asymptomatic parasitemias after the end of the last clinical period. Only one had clinical recurrence after becoming asymptomatic for over a few days. Four representative cases are discussed below and are shown graphically in Figure 2. Parasite counts were made twice weekly or more often, except when the patient was on furlough.

*Patient 1.* White male, age 25, Mediterranean theater. Had had 5 previous attacks beginning July, 1943. Observed attack began October 22, 1944. Paroxysms were quotidian for 22 days when they ceased. No fever or other symptoms, except minimal temperatures on 29th, 30th, and 32nd days, were noted until the 48th and 50th day of observation when temperatures over 104° F. were measured and patient chilled. Patient was then asymptomatic until the 86th day of observation. Light chills were recorded the 86th and 90th day; fever at 103.7°F. and heavy chill on 88th day. Patient was then observed for seven months with no clinical activity; parasite activity was at first continuous during the seven months but short periods during which parasites could not be demonstrated became increasingly frequent toward the end of the observation period. Level of parasitemia became pregressively lower until at the end of observation counts of less than 100 per cmm. were usual (Figure 2).

*Patient 2.* White male, age 29, Pacific theater. Had had eight previous attacks starting in May, 1944. Observed attack began October 22, 1944. Patient had paroxysms at irregular intervals until the 30th day of observation (eight paroxysms during 30-day period), only one of the paroxysms being accompanied by more than 104°F. fever. Patient was clinically inactive until 138th day at which time he requested treatment due to continuous unwell feeling which was most likely neurotic. This patient had a terminal parasitemia at first continuous but punctuated toward the last by long periods during which parasites were not detected in the blood. It is worthy of note that after treatment patient had a further asymptomatic parasitemia two months later which lasted seven days beginning the 200th day of observation.

*Patient 3.* White male, age 25, Pacific theater. Had had ten previous attacks. After observed attack began October 26, 1944, patient had three tertian paroxysms. After the last paroxysm patient became free of parasites on the 15th day of observation. Starting on the 106th day of observation he showed small numbers of parasites (less than 10 per cmm.) for 13 days. Subsequent to this, patient was free of parasites until observation ceased on the 165th day.

*Patient 4.* Colored male, age 26, Mediterranean theater. Had had eight previous attacks. Observed attack began September 7, 1944, and only a single paroxysm was suffered. Patient was with-

out parasites on 6th day of observation but parasites without symptoms appeared on 16th day. Patient had low parasitemia (below 50 per cmm.) until 21st day at which time he went on furlough Subsequently asymptomatic parasitemias appeared for short periods during which low densities

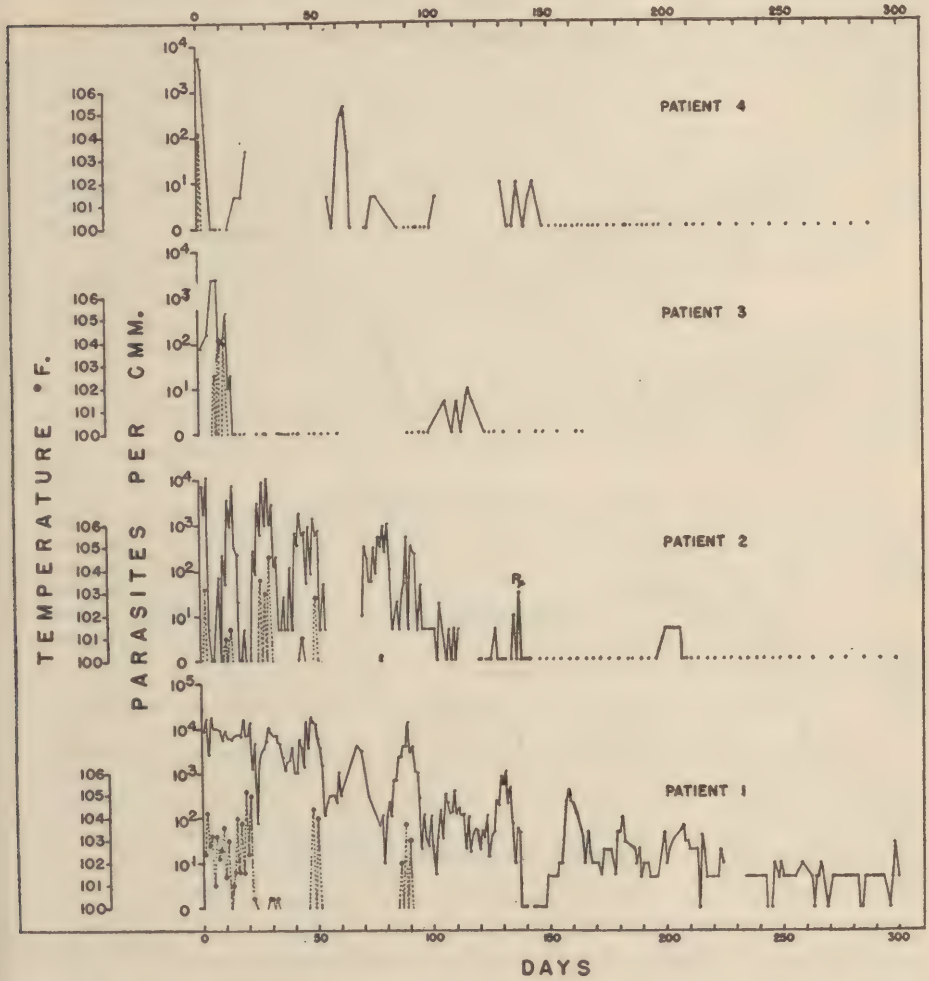



FIG. 2. Pattern of parasite and clinical activity in 4 patients who volunteered to deny themselves treatment. The patients had experienced between 5 and 10 clinical attacks before the beginning of this observation. Blood smears were taken twice weekly or more often except when patient was on furlough, which period is indicated by blank spaces. Dots on base line indicate that no parasites were found on blood smear. The parasite density is shown on a logarithmic scale, viz., $10^1 = 10$, $10^2 = 100$, $10^3 = 1000$, etc.

●——● Parasites per cmm. ○- - - -○ Temperature °F

prevailed. After the last parasites were noted on the 144th day of observation, patient was free of parasites until observation ceased on the 287th day.

Although the group of ten patients is too small to indicate probabilities in a large group, it can be seen that the parasitological behavior of untreated patients varies greatly. Of our ten patients, at least half had extended clinical activity despite the

fact that several had had a large number of previous attacks (this includes the three patients whom it was necessary to treat). The other half of the group, however, had spontaneous clinical remissions after a very small number of paroxysms.

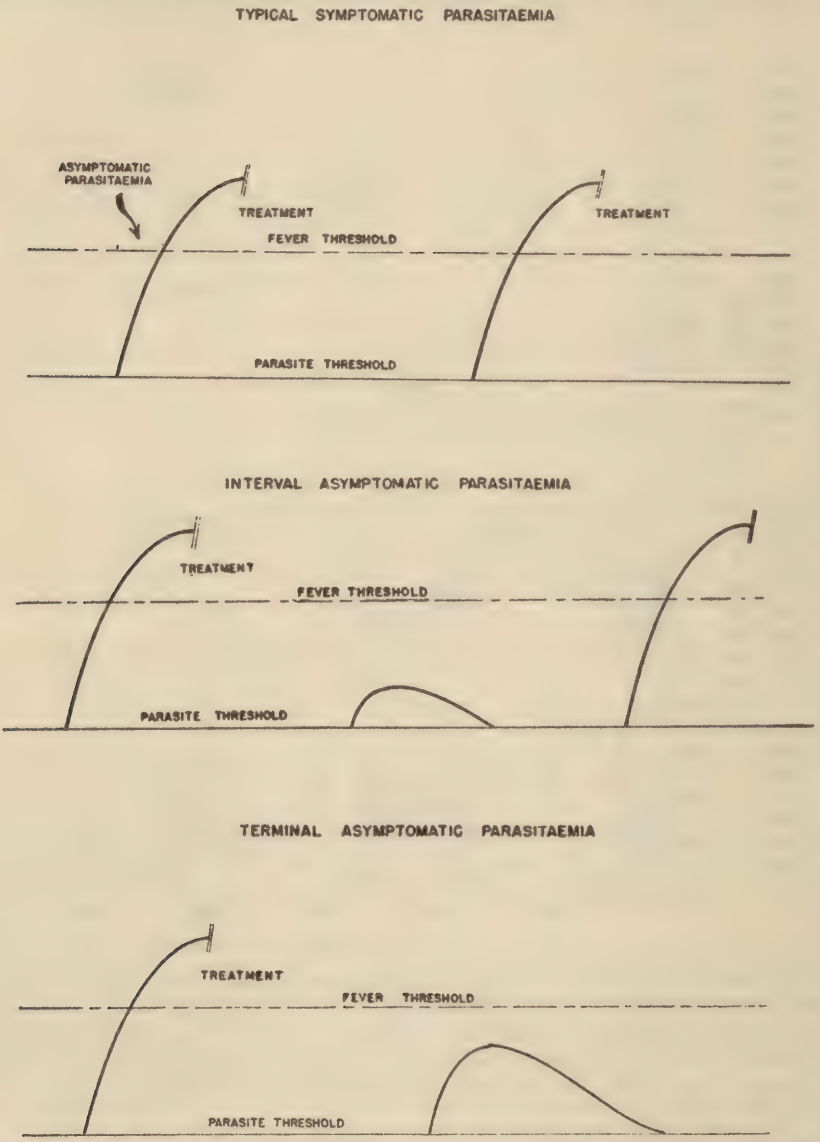



FIG. 3. Characteristic patterns observed in military personnel infected with *Plasmodium vivax* malaria.

Behavior after spontaneous termination of symptoms also was varied. Two of seven patients followed had persistent parasitemias of relatively high level; whereas, five showed only sporadic parasite activity during the terminal observation period.

It is of interest to note that the remittent parasitemia of patient 1 showed maxima about every 22 days (mean of 22.1 ± 0.9 days).

## DISCUSSION

The previous sections have described findings based on the observation of *Plasmodium vivax* in a large group of military patients. It is possible to relate these findings and outline several characteristic patterns of parasitological behavior. It should be emphasized that these patterns were subject to much variation and intercombination.

The most frequent pattern was characterized by treated clinical relapse followed by treated clinical relapse (Figure 3, upper graph). Asymptomatic parasitemia was present only during a few days just preceding the onset of relapse symptoms (preclinical asymptomatic parasitemia). Termination of the disease was sudden; after a last treated clinical episode, no further parasites were found.

A similar pattern increasingly frequent toward the end of the war and subsequently was characterized by a delayed primary attack with a low parasite level which, after treatment, was followed by a variable number of relapses with higher parasite levels. The increased frequency of the delayed primary was probably due to improved atabrine discipline; during the early war days most patients had primary attacks overseas.

A significant proportion (about 25 per cent) of the patients showed a variation of this pattern in which the termination of the disease came as a terminal asymptomatic parasitemia. Other patients aborted symptoms without treatment; these patients also had terminal asymptomatic parasitemias (Figure 3, lower graph).

A small number of patients, about 12 per cent, showed parasite activity between clinical episodes, "interval asymptomatic parasitemias" (Figure 3, middle graph). These parasitemias were usually transient and were characterized by low parasite densities.

Despite the fact that the classification of patients by theater very likely results in the lumping of observations on a number of different strains, several consistent differences between attacks in persons from the Pacific and Mediterranean theaters were noted. Higher parasitemia levels characterized the Mediterranean relapse attacks and gametocyte incidence was significantly greater. As was noted by Most *et al.* (1946) the Mediterranean cases showed a much smaller proportion relapsing. Since Most and his associates studied the same group of patients under consideration here we have not included our observations on relapse. We did attempt to correlate relapse with parasitemia level but found that patients with high and low parasite levels relapsed in similar proportions.

The rate at which the different types of parasitemias infect mosquitoes is considered in an accompanying paper (Eyles, Young and Burgess 1948).

## SUMMARY AND CONCLUSIONS

This report has presented data based on the observation of over 700 patients through more than 1000 individual clinical attacks of malaria. A substantial proportion of these patients were examined by blood smears during the interval between successive clinical attacks and for a 120-day period after the last clinical expression of the disease.

Summarized findings were as follows:

1. About three-quarters of patients undergoing relapse attacks showed parasites in the peripheral blood before the onset of symptoms; this preclinical asymptomatic parasitemia endured on the average 3.5 days.

2. Median parasite level at clinical relapse for Pacific cases was 2952 per cmm. Median parasite level at relapse for Mediterranean cases was significantly higher being 3836 per cmm.

3. No significant difference between parasite level during early clinical relapses and late relapses was found.

4. Patients with high or low parasite densities during one clinical episode tended to have high or low counts, respectively, during a second.

5. Patients with high or low parasite counts at one relapse relapsed again in similar proportions.

6. Male gametocyte incidence was significantly higher in Mediterranean than in Pacific cases.

7. Patients with or without gametocytes during one relapse were likely to be with or without, respectively, during a following relapse attack.

8. Delayed primary attacks from the Pacific theater were found to occur on the average 7 weeks after the discontinuation of suppression.

9. Parasite level at most of the delayed primary attacks was significantly lower than during relapse.

10. Gametocyte incidence during the delayed primary was lower than at relapse but significance could not be demonstrated.

11. Mean interval, without regard to type of the drug used, from the onset of one clinical episode to the onset of a second was 61.1 days. Cumulative frequency study indicated that most relapses had taken place by 120 days.

12. Most patients showed no parasite activity during the interval between clinical attacks. About 12 per cent showed transient, low level, interval asymptomatic parasitemias, occurring on the average about 56 days after the onset of the preceding clinical episode. These parasitemias persisted on the average 12 days, the next clinical relapse following about 24 days later.

13. About 25 per cent of the patients had terminal asymptomatic parasitemias. These occurred on the average 80 days after the onset of the last clinical episode and persisted for an average of 44 days. Parasitemias were remittent or intermittent and levels were much lower than levels that provoked symptoms earlier in the same individuals.

14. Terminal asymptomatic parasitemias of varying intensity persisted in untreated patients after spontaneous termination of symptoms.

15. Malaria parasites were present in the peripheral blood of the Pacific malaria patient 13 per cent of the time and in Mediterranean patients about 10 per cent of the time. In both groups 75 to 80 per cent of the time of parasitemia was asymptomatic.

## REFERENCES

Earle, W. C., and Perez, M. 1932. Enumeration of parasites in the blood of malarial patients. Jour. Lab. Clin. Med., **17**: 1124.

Eyles, D. E., Young, M. D., and Burgess, R. W. 1948. Studies on imported malarias: 8. Infectivity to mosquitoes of asymptomatic *Plasmodium vivax* parasitemias. Jour. Nat. Mal. Soc. (in press).

Moore, J. A., Young, M. D., and Hardman, N. H. 1945. Studies on imported malarias: 2. Ability of California anophelines to transmit malarias of foreign origin and other considerations. Jour. Nat. Mal. Soc., **4**: 307–329.

Most, H., London, I. M., Kane, C. A., Lavieties, P. H., Schroeder, E. F., and Hayman, J. M., Jr. 1946. Chloroquine for treatment of acute attacks of *vivax* malaria. Jour. A. M. A. **131**: 963–967.

Young, M. D., Ellis, J. M., and Stubbs, T. H. 1946a. Studies on imported malarias: 5. Transmission of foreign *Plasmodium vivax* by *Anopheles quadrimaculatus*. Amer. Jour. Trop. Med., **26**: 477–482.

Young, M. D., Stubbs, T. H., Ellis, J. M., Burgess, R. W., and Eyles, D. E. 1946b. Studies on imported malarias: 4. The infectivity of malarias of foreign origin to anophelines of the Southern United States. Amer. Jour. Hyg., **43**: 326–341.

Young, M. D., Stubbs, T. H., Moore, J. A., Ehrman, F. C., Hardman, N. F., Ellis, J. M., and Burgess, R. W. 1945. Studies on imported malarias: 1. Ability of domestic mosquitoes to transmit *vivax* malaria of foreign origin. Jour. Nat. Mal. Soc., **4**: 127–131.

Reprinted from THE JOURNAL OF THE NATIONAL MALARIA SOCIETY
Vol. 7, No. 2, June, 1948

# STUDIES ON IMPORTED MALARIAS

## 8. INFECTIVITY TO *Anopheles quadrimaculatus* OF ASYMPTOMATIC *Plasmodium vivax* PARASITEMIAS[1]

DON E. EYLES, MARTIN D. YOUNG AND R. W. BURGESS[2]

*Division of Tropical Diseases, Office of Malaria Investigations, Colombia, S. C.*

(Received for publication 5 May 1947)

Previous investigations have demonstrated that asymptomatic parasitemias often exist in persons infected with *Plasmodium vivax* returning from tropical military service. Since persons with parasites in the peripheral blood but without symptoms would be more likely to become exposed to mosquitoes than patients in bed or in hospitals, the likelihood of mosquito infection becomes epidemiologically important. The infectivity of such asymptomatic malarias to *Anopheles quadrimaculatus* is the basis of this report.

An accompanying paper in this series (Eyles and Young, 1948) outlines the types of asymptomatic parasitemia encountered in a study of a large group of Army malaria patients. Referring to this paper, asymptomatic parasitemias may occur as follows:

1. Just preceding relapse, parasites may be present in the blood for a period varying from one to several days and are defined as "preclinical parasitemias."
2. During the interval between clinical episodes patients may have parasites without symptoms; these parasitemias are usually transient and low in level and are defined as "interval parasitemias."
3. Following the last clinical expression of the disease, asymptomatic parasitemias may occur as a final phase of the disease in man; these episodes are defined as "terminal parasitemias."

The same patient only rarely had asymptomatic parasitemias of all three types.

### METHODS

Insectary reared *Anopheles quadrimaculatus* mosquitoes varying from 30 to 100 females per lot were fed on volunteer Army patients with asymptomatic parasitemias using the method described by Burgess and Young (1944). These mosquitoes were dissected after sufficient time (about 10 days) had elapsed to allow easy counting of the oocysts, but before sporozoites had reached the salivary glands. If the infections were low in intensity or absent the entire lot was dissected at this time. If the infection was heavier a few mosquitoes were incubated longer to demonstrate that the infection was completed to the glands.

[1] Contribution from the Imported Malaria Studies program of Malaria Investigations, National Institute of Health, and the Office of Malaria Control in War Areas, United States Public Health Service, Columbia, S. C.

[2] Moore General Hospital and Fort Jackson (S. C.) Regional Hospital made the relapsing cases available. To these and especially to Moore General and the South Carolina State Hospital, which also furnished laboratory quarters, we express appreciation. We are also indebted to the Office of The Surgeon General, United States Army, whose active interest made the program possible.

The group of random feedings on symptomatic parasitemias used for comparison was secured in an essentially similar manner.

Parasitological follow-up of the patients was outlined in an accompanying paper (Eyles and Young, 1948). Gametocyte counts were restricted to males because of the fact that most quantitations were made from thick blood films in which female gametocytes are difficult to recognize.

## OBSERVATIONS

*Infectivity of asymptomatic parasitemias.* A total of 118 lots, comprising 2,059 mosquitoes dissected, were fed on 35 patients with asymptomatic parasitemias. Patients were originally chosen at random and repeated feedings on the same patient were made whenever practicable. One patient was fed upon 57 times; one patient, twelve times; one, six times; one, four times; one, three times; six, twice. The other 24 patients were fed upon a single time.

Of the total, 28.0 per cent of the lots were infected and 11.6 per cent of the mosquitoes in all lots were infected. Of the individual patients, about 34 per cent at one time or another infected mosquitoes while asymptomatic.

Feedings were made on patients from both the Mediterranean and Pacific theaters; the results being similar, these are not discussed separately.

Table 1 summarizes these feedings by the type of asymptomatic parasitemia present. It will be noted that infections resulted from each type in some lots, which would indicate that malaria patients are potential infectors whenever parasites are present.

Eight of 21 lots (38.1 per cent) fed on patients with preclinical asymptomatic parasitemia were infected; however, due to the relatively small number of lots, the error of this sample is large (S.E. = 10.6). A little over one-fifth of all mosquitoes applied became infected and the mean number of cysts per infected gut was relatively high (27.4).

Patients with interval asymptomatic parasitemia were fed upon only three times. Two lots (both fed on the same patient) were infected. The sample is admittedly small but it demonstrates that these interval asymptomatic parasitemias are sometimes infective. The percentage of all mosquitoes infected was high (45.1), but the number of cysts per infected gut was relatively low (6.4).

The largest group of feedings was made on patients with terminal asymptomatic parasitemia. About one-quarter of 94 lots fed on such patients became infected. Infections were generally of low intensity (7.5 per cent of 1,555 mosquitoes infected with a mean of 9.4 cysts per infected gut).

Serial feedings were made on several patients with terminal asymptomatic parasitemias. In each case mosquito infection rates were highest early during the course of the parasitemia when parasite counts were also highest. Figure 1, based on 51 daily feedings starting on the first day of the asymptomatic parasitemia upon a single patient, illustrates this fact typically. It is shown that mosquitoes were infected 13 times during the first 42 days but not at all thereafter. There were six additional feedings on this patient between the 85th and 134th day of asymptomatic parasitemia; no infections in mosquitoes resulted.

Except for the small group of feedings on patients with interval asymptomatic parasitemia, the largest proportion of infected lots was in the group fed on patients

TABLE 1

*Mosquito infections resulting from feedings on patients with asymptomatic* Plasmodium vivax *parasitemias*

| | TYPE OF PARASITEMIA | | | |
|---|---|---|---|---|
| | Preclinical | Interval | Terminal | All |
| Number of patients | 17 | 2 | 16 | 35 |
| Mosquito lots fed | 21 | 3 | 94 | 118 |
| Mosquito lots infected | 8 | 2 | 23 | 33 |
| Per cent lots infected | 38 ± 11 | 66 ± 28 | 25 ± 4 | 28 ± 4 |
| Number of mosquitoes dissected | 433 | 71 | 1,555 | 2,059 |
| Per cent mosquitoes infected | 21.2 | 45.1 | 7.5 | 11.6 |
| Mean number of cysts per infected gut | 27.4 | 6.4 | 9.4 | 14.8 |

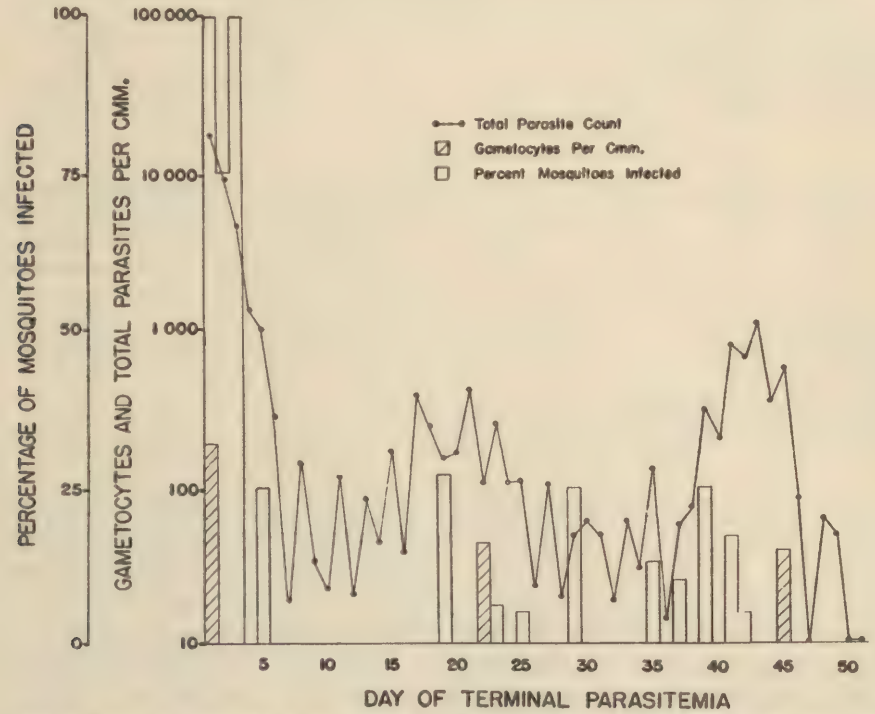



FIG. 1. *P. vivax* infections in *A. quadrimaculatus* resulting from 51 consecutive daily feedings upon a single patient. Feedings were started on the first day of a terminal asymptomatic parasitemia. Not shown in the figure are six additional feedings between the 85th and 134th day of asymptomatic parasitemia from which no mosquitoes were infected.

with preclinical parasitemia. The difference between the proportion infected by the latter (38.1 per cent) and the proportion infected by the terminal asymptomatic para-

sitemias (24.5 per cent) would occur by chance one in four times, all factors being equal. The fact that more cysts per infected gut and a larger proportion of infected mosquitoes were found in the individual lots fed on patients with preclinical parasitemia may indicate a greater degree of significance than was disclosed by the statistical method used.

*Relative infectivity of asymptomatic and symptomatic parasitemias.* Table 2 compares the entire group of 118 feedings on asymptomatic patients with a series of 114 random feedings on clinical cases. The lots fed on the clinical cases were more highly infected, not only as to the percentage of lots infected (49.1 per cent against 28.0 per

TABLE 2

*Comparison of mosquito feedings on clinical cases versus feedings on patients with asymptomatic* Plasmodium vivax *parasitemias*

| | CLINICAL CASES | ASYMPTOMATIC CASES |
|---|---|---|
| Mosquito lots fed | 114 | 118 |
| Mosquito lots infected | 56 | 33 |
| Per cent lots infected | 49.1 ± 4.7 | 28.0 ± 4.1 |
| Number mosquitoes dissected | 3,853 | 2,059 |
| Per cent mosquitoes infected | 24.9 | 11.6 |

TABLE 3

*Comparison of mosquito feedings during clinical episodes and during terminal asymptomatic parasitemia on the same patient.* Plasmodium vivax

| | CLINICAL PERIOD | ASYMPTOMATIC PERIOD |
|---|---|---|
| Mosquito lots fed | 3 | 57 |
| Mosquito lots infected | 3 | 14 |
| Per cent lots infected | All | 24.6 |
| Number mosquitoes dissected | 68 | 782 |
| Per cent mosquitoes infected | 87 | 6.1 |

cent), but also as to percentage of mosquitoes infected. Mean number of cysts per infected gut was not recorded for all of the symptomatic lots but when noted were higher than in the asymptomatic group. Statistical comparison based on the standard errors of the percentages of lots infected demonstrates a high degree of significance to the difference ($p = .0018$).[3] It should be borne in mind that 57 of the asymptomatic feedings were made on a single patient. Three additional feedings were made on this same patient when he was ill. Table 3 compares these feedings; despite the

[3] A more nearly random statistical comparison was made between the symptomatic feedings as given here and the first feeding on each of the 35 asymptomatic volunteers. Of the asymptomatic cases, 34.3 ± 8.0 per cent were infected (S.E. 8.024) against 29.1 ± 4.7 per cent of the symptomatic. Probability in this case is 0.112 which is above the usual level of significance, but the difference appears more significant when it is considered that the individual symptomatic lots had over twice as many mosquitoes infected.

small number of feedings while this patient was ill, the lower infection values when the patient was asymptomatic are highly significant ($p = .0002$).

*Parasite and gametocyte density as related to infectivity.* Table 4 compares the mean total parasite and male gametocyte densities of the three types of asymptomatic parasitemia with the symptomatic at time of feeding. This tabulation also indicates the proportion of patients with patent gametocytes and repeats the proportion of mosquito lots infected. Infection in the mosquitoes is seen to correspond well both with the percentage of patients with male gametocytes and the mean male gametocyte density.

In the case of the preclinical asymptomatic parasitemia we believe the group of patients upon whom mosquitoes were fed to be representative of the larger number of patients whose cases were followed parasitologically. Eyles and Young (1948) state that during the preclinical phase parasitemias rise steadily from scarcely detectable

TABLE 4

*Parasite levels at time of feeding and proportion of smears with male gametocytes compared with proportion of mosquito lots infected.* Plasmodium vivax

| TYPE OF PARASITEMIA | MEAN COUNT PER CMM. | | PER CENT SMEARS WITH GAMETOCYTES | PER CENT MOSQUITO LOTS INFECTED |
|---|---|---|---|---|
| | Total parasites | Male gametocytes | | |
| Preclinical asymptomatic | 1,763 | 25 | 19.0 | 38.1 |
| Interval asymptomatic | 163 | 78 | 66.7 | 66.7 |
| Terminal asymptomatic | 606 | 4 | 8.5 | 24.5 |
| All asymptomatic | 800 | 10 | 11.9 | 28.0 |
| Symptomatic* | 6,577 | 46 | 50.6 | 49.1 |

* These data are from 77 of the 114 symptomatic feedings; only those cases in which both total count and male gametocyte count were known were tabulated.

levels to levels sufficiently high to provoke symptom response (mean fever threshold count at relapse observed by Eyles and Young to be 6300 per cmm.). The mean total parasite level observed in our group of patients would be near that which would be expected in a random sample. The mean male gametocyte level observed in the group of patients upon whom mosquitoes were fed is also such as might be expected (gametocyte count at relapse 46 per cmm.).

We do not believe, however, that the group of three patients with interval asymptomatic parasitemias upon whom mosquitoes were fed to be representative. Table 5 presents data based on the study of 24 patients with this type parasitemia and it can be seen that the incidence of gametocytes and the ratio of male gametocytes to total parasites is much lower in the large group than in the group upon whom mosquitoes were fed. Consequently, the proportion of lots infected on these patients is almost certainly misleadingly large. Eyles and Young (1948) state that total parasite level in this type of asymptomatic parasitemia is low; lower, in fact, than in the other types of asymptomatic parasitemias. This fact together with the fact that no great dissimilarity in the ratio of gametocytes to total parasites exists in this group of patients

when symptomatic and when asymptomatic is further evidence of the non-representative nature of our group of volunteers.[4]

The group of patients with terminal asymptomatic parasitemias upon whom mosquitoes were fed is apparently quite representative of the larger group studied parasitologically. Table 5 analyzes data on 76 patients and the proportion of smears with gametocytes in this group is very similar to the proportion in the smears made coincident with feeding (6.3 per cent as compared with 8.5 per cent).

Eyles and Young (1948) showed that parasite levels during the terminal asymptomatic parasitemia are seldom higher than one-quarter to one-half of those noted during the clinical attack, the level gradually decreasing after onset of parasitemia. The fact that in the same patients only about one-third as many smears with gametocytes evident were found when terminally asymptomatic as when clinically ill is undoubtedly due to the lower parasite levels as the ratio of male gametocytes to total parasites was similar during parasitemias with and without symptoms (table 5).

TABLE 5

*The incidence of male gametocytes in interval and terminal asymptomatic parasitemias compared with parasitemias accompanied by symptoms in the same patients.* Plasmodium vivax

| | CLINICAL PARASITEMIA | INTERVAL ASYMPTOMATIC PARASITEMIA | CLINICAL PARASITEMIA | TERMINAL ASYMPTOMATIC PARASITEMIA |
|---|---|---|---|---|
| Number of patients | 24 | 24 | 76 | 76 |
| Total number of smears | 142 | 118 | 326 | 907 |
| Per cent smears with gametocytes | 19.0 | 6.8 | 21.2 | 6.3 |
| Ratio of number of gametocytes to total parasites | 0.0048 | 0.0107 | 0.0083 | 0.0081 |

Tables 6 and 7 compare infection rates in the mosquitoes in the symptomatic group with those in the asymptomatic group at different male gametocyte and total parasite levels. It will be noted that in both the asymptomatic and the symptomatic groups at least a fifth of the lots were infected when no male gametocytes were discerned (less than 10 per cmm.). In spite of apparent dissimilar infection rates, in no case at the various levels of male gametocyte and total parasite density are the differences between the corresponding proportions of lots of mosquitoes infected on asymptomatic and symptomatic parasitemias statistically significant. Simply stated, the evidence indicates that similar parasite or gametocyte numbers are equally effective in infecting mosquitoes in both the asymptomatic and symptomatic patients.

From the preceding tables (4, 6, and 7) it would appear that infection in the mosquito is positively correlated both with total parasite count and male gametocyte count. In order to determine which count would be the more reliable criterion to use in estimating potential infectivity of asymptomatic patients the relationship between intensity of infection in the mosquitoes and parasite or male gametocyte density was measured by means of the Pearsonian coefficient of correlation (r) for the entire group

[4] Such dissimilarity as exists in the ratios is due to the inclusion of a single high gametocyte count in one asymptomatic patient, this patient being the one upon whom two lots of mosquitoes were fed.

of asymptomatic feedings. For the mosquitoes an index of intensity of infection was expressed in such a manner as to take into regard both the percentage of mosquitoes infected in each lot and the number of cysts per infected gut (infection index = per cent mosquitoes infected × mean number of cysts per infected gut).

Coefficient of correlation (r) between male gametocyte density and infection index was found to be about +0.7. By means of the Z transformation it was found that this value was not significantly different from a hypothetical r value of 1.0 ($p = 0.22$).

TABLE 6

*Mosquito infection rate (percentage of lots infected) in relation to male gametocyte count.* Plasmodium vivax

| MALE GAMETOCYTES PER CMM. | PER CENT OF LOTS INFECTED | | | |
|---|---|---|---|---|
| | Asymptomatic group | | Symptomatic group | |
| | Lots fed | Per cent infected | Lots fed | Per cent infected |
| Less than 10* | 104 | 20.0 | 50 | 22.0 |
| 10–20 | 8 | 75.0 | 14 | 42.9 |
| Over 20 | 6 | 100.0 | 50 | 78.0 |

* No gametocytes found in 0.1 cmm. of blood.

TABLE 7

*Mosquito infection rate (per cent of lots infected) in relation to total parasite count.* Plasmodium vivax

| TOTAL PARASITE COUNT PER CMM. | PER CENT OF LOTS INFECTED | | | |
|---|---|---|---|---|
| | Asymptomatic group | | Symptomatic group | |
| | Lots fed | Per cent infected | Lots fed | Per cent infected |
| 1–50 | 46 | 6.5 | 3 | 0.0 |
| 51–250 | 31 | 19.4 | 1 | 0.0 |
| 251–1250 | 26 | 34.2 | 9 | 22.2 |
| 1251–6250 | 12 | 75.0 | 33 | 36.4 |
| 6251 and over | 3 | 100.0 | 27 | 66.7 |

Coefficient similarly calculated for total parasite density and infection index for the same feedings was about +0.5. This value was compared with the value of +0.7 found above by means of the Z transformation and the two were significantly different ($p = .0005$).

From the above it would appear that, despite the fact that infection resulted in over one-fifth of the instances in which mosquitoes were fed on patients in which gametocytes were sub-patent, the best estimate of the potential infectivity of a patient can be made on the basis of the male gametocyte count. It should be pointed out that correlations on the basis of either total or female gametocyte count were not attempted.

In those cases in which no gametocytes were observed, infection in the mosquitoes

was positively correlated with the total parasite count ($r = + 0.3$), but not to a degree sufficient to estimate accurately the likelihood of infection.

## DISCUSSION

As was stated in introducing this paper, persons with asymptomatic malaria parasitemias are more likely to expose themselves to mosquitoes than ill patients. It has been recognized for some time that returning servicemen are at times asymptomatic carriers of *Plasmodium vivax*, but to this time neither the incidence of the asymptomatic parasitemias nor the degree to which they can infect mosquitoes has been adequately estimated.

In an accompanying paper Eyles and Young (1948) have demonstrated that a significantly large proportion of servicemen with relapsing malaria will have these asymptomatic periods. It was shown that two-thirds of a large group of individuals had detectable numbers of parasites for several days prior to the onset of relapse symptoms; twelve per cent showed small numbers of parasites during the interval between clinical episodes; and in 25 per cent of the individuals observed the termination of the disease came as an asymptomatic parasitemia which often endured for long periods of time. It was shown also that asymptomatic parasitemias prevail several times as long as symptomatic parasitemias.

Since a large number of ex-servicemen will carry on their normal activities while still harboring parasites, the assessment of the hazard they will constitute to the community is of vital importance.

Our evidence shows that, qualitatively, the malaria patient can infect mosquitoes throughout the duration of the disease, whenever parasites are present in the peripheral blood. Our evidence shows, furthermore, that the degree to which mosquitoes are infected is low during asymptomatic periods, being proportional to the male gametocyte count and the total parasite count which are also low as compared with the symptomatic periods.

These findings, based upon 118 feedings on asymptomatic patients, are not in accord with those of Watson (1945a, 1945b) who found higher infection rates in 7 lots of mosquitoes fed on asymptomatic patients than in a larger number of lots fed on patients with symptoms. As was pointed out by Christianson *et al.* (1946), comparison with Watson's work is difficult because of the fact that Watson did not state the percentage of patients who actually infected mosquitoes.

We found a lower incidence of gametocytes in smears made upon asymptomatic patients than in smears made on ill patients. Similar ratios of gametocytes to total parasites were found in asymptomatic and symptomatic parasitemias, the lower incidence of gametocytes in asymptomatic smears probably being due to the much lower parasite levels. The higher incidence of gametocytes in fever-free patients reported by Christianson *et al.* (1946) is not statistically significant for the number of cases and smears they studied.

Taking all factors into consideration, it would appear that the low infectivity to mosquitoes of asymptomatic malaria is offset by the frequent occurrence and relatively long prevalence of these symptomless parasitemias. In areas very favorable to mosquito transmission, the asymptomatic patient would be a definite hazard.

## SUMMARY AND CONCLUSIONS

1. A total of 118 lots of *Anopheles quadrimaculatus* comprising 2,059 mosquitoes dissected were fed on 35 asymptomatic carriers of foreign *Plasmodium vivax*. The results demonstrated that malaria patients are potentially infective to mosquitoes whenever parasites are present.

2. Infection was significantly lower in asymptomatic cases than in a random group of 114 feedings comprising 3,853 mosquitoes dissected fed on patients with symptoms, not only as to percentage of lots infected but also as to percentage of mosquitoes infected.

3. Infection in the mosquitoes was positively correlated with the number of male gametocytes present, but about one-fifth of the lots, both of the asymptomatic and symptomatic group, were infected when fewer than 10 male gametocytes per cmm. were present.

4. Infection in the mosquitoes fed on asymptomatic and symptomatic carriers varied directly in intensity with the total parasite count, the lower parasite counts of the asymptomatic cases resulting in lower infection values.

5. Male gametocytes were observed to have a lower incidence in smears taken on asymptomatic patients, the lower incidence being due to lower levels of parasitemia.

6. No evidence was found to indicate an increased production of gametocytes during asymptomatic periods. Similar ratios of male gametocytes to total parasites held for asymptomatic and symptomatic parasitemias.

7. On the basis of these results, it is concluded that patients showing asymptomatic parasitemias of foreign *vivax* malarias can infect mosquitoes but at a lower rate than those with symptomatic parasitemias. However, as the asymptomatic patient is more likely to be exposed to malaria vectors than the ill patient, the overall hazard of the asymptomatic malaria carrier may be as great or greater than that of the one clinically ill.

## REFERENCES

Burgess, R. W., and Young, M. D. 1944. Methods of handling and feeding *Anopheles quadrimaculatus* Say upon malarious patients. Jour. Nat. Mal. Soc., **3**: 241–247.

Christianson, H. B., Gordon, H. H., Daniels, W. B., and Lippincott, S. W. 1946. Afebrile parasitemia in imported *vivax* malaria. Amer. Jour. Publ. Hlth., **36**: 759–761.

Eyles, D. E., and Young, M. D. 1948. Studies on imported malarias: 7. The parasitological pattern of relapsing *Plasmodium vivax* in military patients. Jour. Nat. Mal. Soc., **7**: 23–37.

Watson, R. B. 1945a. Implications of the importation of malaria by personnel of the armed forces. Jour. Tenn. State Med. Assn., **38**: 13–18.

Watson, R. B. 1945b. On the probability of soldiers with Pacific *Plasmodium vivax* malaria infecting *Anopheles quadrimaculatus*. Jour. Nat. Mal. Soc., **4**: 183–188.

*Made in United States of America*

Reprinted from THE JOURNAL OF THE NATIONAL MALARIA SOCIETY
Vol. 7, No. 2, June, 1948

# STUDIES ON IMPORTED MALARIAS

## 9. THE COMPARATIVE SUSCEPTIBILITY OF *Anopheles quadrimaculatus* AND *Anopheles maculipennis freeborni* TO FOREIGN *Vivax* MALARIA[1,2]

MARTIN D. YOUNG AND ROBERT W. BURGESS

*Office of Malaria Investigations, Division of Tropical Diseases, National Institute of Health, Columbia, S. C.*

(Received for publication 12 September 1947)

*Anopheles quadrimaculatus* and *A. m. freeborni* are considered to be the principal malaria vectors in the United States of America. Earlier reports (Moore *et al.*, 1945; Young *et al.*, 1946) have shown that these species of mosquitoes were susceptible to and transmitted foreign *Plasmodium vivax* malaria relapsing in returned military personnel.

The object of this report is to compare the susceptibility of these species of mosquitoes to foreign *vivax* malaria.

### METHODS

The procedures followed have been described earlier (Burgess and Young, 1944; Moore *et al.*, 1945). In general, they consisted in feeding mosquitoes (preferably 100 or more of each species) simultaneously upon a patient, incubating the mosquitoes in a temperature-controlled insectary (74°–78°F.), and dissecting the mosquitoes at intervals to determine the infection rates. To compare the number of oocysts, counts were made from the 7th day of incubation until sporozoites appeared in the salivary glands. Oocyst counts made before or after this period were not used for comparison.

The Q-1 strain of *A. quadrimaculatus* and the F-1 strain of *A. m. freeborni* were used. The mosquitoes were applied to soldiers with clinical relapsing foreign *vivax* malaria or to neurosyphilitic patients with primary attacks of foreign *vivax* malaria which had been induced either by transfer of infected blood or the bites of infected mosquitoes.

The malarias had originated presumably in the following areas: Solomon Islands, New Hebrides Islands, New Guinea, Tunisia, Liberia, Trinidad, and the China-Burma-India theater.

### OBSERVATIONS

About 50 feedings were made. Only those (24) in which either one or both species of mosquitoes became infected are shown in the tabulations (table 1).

[1] Contribution from the Imported Malaria Studies program of the Division of Tropical Diseases, National Institute of Health, and the Office of Malaria Control in War Areas, United States Public Health Service, Columbia, S. C.

[2] The Fort Jackson (S. C.) Regional Hospital made relapsing malaria cases available and the active interest of the Office of the Surgeon General, U. S. Army, made the program possible. The South Carolina State Hospital furnished laboratory quarters. To these we express our appreciation.

Measured by the percentage of mosquitoes which were infected, *A. m. freeborni* was significantly more susceptible than *A. quadrimaculatus* to the malaria from the standpoints of (a) relapsing infections in the soldiers (2.4 S.E.), (b) the primary infections in the neurosyphilitic patients (2.7 S.E.), or (c) the total of both (3.6 S.E.). In 4 instances where the *A. quadrimaculatus* were not infected, the *A. m. freeborni* showed the following infection percentages: 18, 7, 3, and 5. In 20 of the 24 feedings, a higher percentage of *A. m. freeborni* than *A. quadrimaculatus* was infected.

To further compare the susceptibility the oocysts on the infected mosquito guts were counted. The *A. m. freeborni* rather consistently had more oocysts per infected gut than did the *A. quadrimaculatus*, and the mean was higher in the former (18.7 as compared to 15.2).

TABLE 1

*Comparison of resulting infections when* Anopheles quadrimaculatus *and* A. m. freeborni *were fed simultaneously upon foreign* vivax *malaria*

The malarias were either relapsing infections in returned soldiers or primary infections induced in white neurosyphilitic patients.

| PATIENT SOURCE | NUMBER OF FEEDINGS | *A. quadrimaculatus* | | | | *A. m. freeborni* | | | | TOTAL MOSQUITOES | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mosquitoes | | Infected guts | | Mosquitoes | | Infected guts | | | |
| | | Dissected | Per cent infected* | Number of guts | Average number of oocysts | Dissected | Per cent infected* | Number of guts | Average number of oocysts | Per cent infected* | S.E.† |
| Soldiers | 18 | 735 | 40.1 | 132 | 18.6 | 625 | 46.6 | 155 | 22.2 | 43.1 | 2.4 |
| Neurosyphilitics | 6 | 186 | 60.2 | 64 | 8.2 | 205 | 71.2 | 69 | 10.9 | 66.0 | 2.7 |
| Total | 24 | 921 | 44.2 | 196 | 15.2 | 830 | 52.7 | 224 | 18.7 | 48.2 | 3.6 |

* An infected mosquito is one showing oocysts, sporozoites, or both.

† Standard error between the means of *A. quadrimaculatus* and *A. m. freeborni*.

## DISCUSSION

It had been noticed in previous work that *A. m. freeborni* showed a higher infection rate, 42.4 per cent (Moore *et al.*, 1945), than *A. quadrimaculatus*, 30.8 per cent (Young *et al.*, 1946), when fed upon relapsing foreign *vivax* malarias. However, as these species of mosquitoes had been tested in separate laboratories and with different patients, it was not certain that the tests were comparable. Consequently, the present work was performed where the 2 mosquito species were tested under identical conditions.

Considering the proportion of mosquitoes becoming infected, and the number of oocysts per infected gut, it appears that *A. m. freeborni* is more susceptible than *A. quadrimaculatus* to foreign *vivax* malaria.

Previously, 4 anophelines of the southern United States, viz., *A. punctipennis*, *A. quadrimaculatus*, *A. pseudopunctipennis pseudopunctipennis*, and *A. albimanus*, were compared as to their relative susceptibility to foreign *vivax* malaria (Young *et al.*,

1946). *A. quadrimaculatus* was used as the standard for comparison in the above tests as well as in the tests with *A. m. freeborni*; therefore, it is possible to assign relative values. Giving *A. quadrimaculatus* a value of 100, the relative values of the other species are shown in table 2. Theoretically one might draw up an evaluation showing what percentage of each species might be expected to become infected in the same situation where the most susceptible species showed a 100 per cent infectivity (table 2).

Also shown in table 2 are the standard errors between the means of the different species tested and *A. quadrimaculatus*.

*A. m. freeborni* appears to be significantly more susceptible than *A. quadrimaculatus*, while *A. p. pseudopunctipennis* and *A. albimanus* are significantly less susceptible. *A punctipennis* appears to have about the same susceptibility as *A. quadrimaculatus*,

TABLE 2

*Comparison of susceptibility to foreign* vivax *malaria of 4 anopheline species to* A. quadrimaculatus *showing the standard errors between the means, the relative numerical evaluation, and the theoretical percentage of infection where the most susceptible species would show a 100 per cent infectivity*

| | STANDARD ERROR* | RELATIVE EVALUATION | THEORETICAL PERCENTAGE EVALUATION |
|---|---|---|---|
| *A. m. freeborni* | 3.6 | 119 | 100 |
| *A. punctipennis* | 0.1 | 102 | 86 |
| *A. quadrimaculatus* | | 100 | 84 |
| *A. p. pseudopuntipennis* | 5.1 | 41 | 35 |
| *A. albimanus* | 20.7 | 2 | 2 |

* Standard error between means of particular species and *A. quadrimaculatus*. The means for *A. punctipennis*, *A. p. pseudopunctipennis*, and *A. albimanus* were given in a previous report (Young *et al.*, 1946).

but the number of mosquitoes involved were small, viz., 46 and 83 respectively, so that the test might not be adequate.

## SUMMARY AND CONCLUSIONS

*Anopheles quadrimaculatus* and *A. m. freeborni* were applied simultaneously to patients with either naturally acquired or induced foreign *Plasmodium vivax* malaria. The malarias had originated from widely separated areas.

*A. m. freeborni* showed more oocysts per infected gut and a significantly higher percentage of mosquitoes infected than *A. quadrimaculatus*.

It is concluded that *A. m. freeborni* is more susceptible to these foreign malarias than *A. qudrimaculatus*.

The theoretical relative evaluation of susceptibility to foreign *vivax* malaria of 5 species of anophelines, using the most susceptible as 100, would be: *A. m. freeborni*, 100; *A. punctipennis*, 86; *A. quadrimaculatus*, 84; *A. p. pseudopunctipennis*, 35; and *A. albimanus*, 2. The differences between the various species and *A. quadrimaculatus* were significant for except *A. punctipennis*.

## REFERENCES

Burgess, R. W., and Young, M. D. 1944. Methods of handling and feeding *Anopheles quadrimaculatus* Say upon malarious patients. Jour. Nat. Mal. Soc., **3**: 241–247.

Moore, J. A., Young, M. D., Hardman, N. F., and Stubbs, T. H. 1945. Studies on imported malarias: 2. Ability of California anophelines to transmit malarias of foreign origin and other considerations. Jour. Nat. Mal. Soc., **4**: 307–329.

Young, M. D., Stubbs, T. H., Ellis, J. M., Burgess, R. W., and Eyles, D. E. 1946. Studies on imported malarias: 4. The infectivity of malarias of foreign origin to anophelines of the Southern United States. Amer. Jour. Hyg., **43**: 326–341.

Reprinted from THE JOURNAL OF THE NATIONAL MALARIA SOCIETY
Vol. 7, No. 3, September, 1948

# STUDIES ON IMPORTED MALARIAS

## 10. AN EVALUATION OF THE FOREIGN MALARIAS INTRODUCED INTO THE UNITED STATES BY RETURNING TROOPS[1]

MARTIN D. YOUNG, DON E. EYLES, AND ROBERT W. BURGESS

*Division of Tropical Diseases, National Institute of Health, Columbia, S.C.*

(Received for publication 10 June 1948)

Early during World War II, it became obvious that military personnel would bring back malaria infections acquired in foreign countries. Therefore, it was necessary to learn as much as possible about these foreign malarias and particularly how they would adapt themselves to conditions here. To determine this and to gather other information, the "Imported Malaria Studies" program was established with the cooperation of the Army, Navy, and U. S. Public Health Service.

Various phases of the problem have been reported in the first nine papers of this series (Young, *et al.* 1945, 1946; Moore, *et al.* 1945; Young, Stubbs and Ellis, 1946; Young, Ellis and Stubbs, 1946; Hardman, 1947; Eyles and Young, 1948; Eyles, Young and Burgess, 1948; and Young and Burgess, 1948). This report will summarize the findings of the entire program, correlate these with other information, and present conclusions based upon the data obtained.

### METHODS

Studies on returned military personnel with foreign malaria were carried on cooperatively in 10 Army and Navy hospitals. There were eight coöperating state, Veterans', and private hospitals which used the foreign malarias for the treatment of neurosyphilis.

To test the susceptibility of mosquitoes, they were fed upon relapsing military personnel and upon neurosyphilitic patients with induced malarias. From these infections, parasitological studies were also made. Foreign malarias were found to be useful as therapeutic agents against neurosyphilis. By inducing these malarias in neurosyphilitic patients, certain strains were propagated which enabled more specific study of host-parasite relationship of the parasite in man and mosquito as well as making available a Pacific strain of malaria (Chesson *vivax*) for the large scale testing of new drugs. The details of the procedures used have been specified in the preceding papers of this series.

During the course of this program, of about $3\frac{1}{2}$ years duration, mosquitoes were reared at the rate of one million per year. The majority of these were *Anopheles quadrimaculatus* and *A. maculipennis freeborni*, which are considered to be the principal vectors of malaria in the South and West, respectively. Other species tested in smaller numbers were *A. m. occidentalis*, *A. p. franciscanus*, and *A. pencitpennis* from

[1] Contribution from the Imported Malaria Studies program of the Division of Tropical Diseases, National Institute of Health, and the Office of Malaria Control in War Areas, United States Public Health Service, P. O. Box 1344, Columbia, S. C.

the West Coast; and *A. punctipennis*, *A. p. pseudopunctipennis*, and *A. albimanus* from the South.

About 50,000 mosquitoes were fed upon military personnel with malaria and of these some 15,000 were dissected to determine infection rates. Additional mosquitoes were fed upon induced cases of foreign malaria.

Over 1,000 military personnel with relapsing malaria were observed to collect various types of data. Approximately another 1,000 patients, either neurosyphilitic patients or volunteers, have been given these foreign malarias.

The data from the above form the basis of the papers in this series.

## OBSERVATIONS

Of the foreign malaria cases examined, none was *Plasmodium malariae* (quartan malaria).

There were eight cases of *P. falciparum* found. One of these from Guadalcanal infected 1 out of 28 *A. m. freeborni;* one *P. falciparum* from Africa infected 2 out of 10 *A. quadrimaculatus*. Six feedings on the other *P. falciparum* cases did not show any mosquitoes infected.

These data indicate only that foreign *P. falciparum* can infect our mosquitoes.

*P. vivax* was the important malaria studied because most of the infections in returning soldiers were of this species and it was the type responsible primarily for the relapses. The remaining part of this report will concern observations on this species.

## THE PARASITE

*Morphology.* Careful observations were made to determine whether the foreign *vivax* parasites might show morphological differences which would be specificially characteristic and diagnostic. In addition to our own observations, Miss Aimee Wilcox, Associate Parasitologist of the National Institute of Health, examined smears from 71 different infections and to her we express our especial gratitude.

Even though the *vivax* infections originated from widely separated areas such as the South Pacific, Caribbean, and Mediterranean, no consistent morphological differences were observed.

On a few occasions, in two different *vivax* infections from New Guinea, forms were found which resembled *P. ovale.* The occurrence of these forms were transient, however, and it is believed that they were abnormal forms of *P. vivax.* These observations will be reported in detail elsewhere.

*Strains.* Lacking specific differential morphological characters, the indication of strain differences was secured by such criteria as immunity, characteristics of the primary infection, and relapse patterns.

Little heterologous immunity was shown between *vivax* infections from the South Pacific, China-Burma-India theatres and from the United States (Young, *et al.* 1947). In fact, one infection originating from New Guinea exhibited little immunity to another infection from the same area, suggesting multiplicity of strains in small areas.

Differences in the prepatent and incubation periods of malarias from the Pacific

and Mediterranean were demonstrated, the latter having the shorter periods (Young, *et al.* 1947).

Differences in the lengths of the asexual cycles were demonstrated in strains from the Mediterranean, American, and Pacific areas (Young, *et al.* 1947). In fact, strains from the same localities (New Guinea and Guadalcanal) in the Pacific area showed different periodicities.

Following treatment with certain drugs, a Pacific *vivax* (Chesson strain) isolated by us (Ehrman, *et al.* 1945) tends to relapse after a short latent period as contrasted to a long latent period as exhibited by an American strain (Coatney, *et al.* 1947, 1948a; Whorton, *et al.* 1947).

Because of the complexity of securing such data as the above, the differentiation of strains is slow. However, the results so far indicate that among the foreign infections brought into this country, there must be many different and distinct strains. As these are propogated in nature they are added to the various strains already indigenous.

## THE PARASITE IN MAN

*Susceptibility of Americans to foreign malarias.* Approximately 1,000 white patients have been bitten by native anophelines infected with foreign malaria. Practically all of them were susceptible to these malarias as evidenced by a resulting infection, the failures being on the order of five per cent or less.

American Negroes are quite resistant to these foreign *vivax* malarias, from both the Pacific and Mediterranean areas. In one controlled study (Young, Ellis and Stubbs 1946), 31 per cent of the Negroes bitten by infected mosquitoes became infected as compared to 95 per cent of the white patients.

Thus, it appears that white natives of this country are almost universally susceptible to the foreign *vivax* malarias. The negroes are quite resistant, just as they appear to be to native *vivax* malarias.

*The primary infections induced in neurosyphilitic patients.* When these malarias were transmitted to patients by the bites of infected mosquitoes, usually 5 to 10, the prepatent and incubation periods (average 12.1 and 13.7 days respectively) in the Mediterranean strains were shorter than the same periods (average 13.1 and 14.4 days respectively) in the Pacific strains Young, *et al.* 1947). A domestic strain, St. Elizabeth, showed longer prepatent and incubation periods (15.4 and 16.9 days respectively) than the foreign strains (unpublished data).

As indicated above, the parasites were usually present in the blood stream before the appearance of fevers but at the first fever of 100°F. the number of parasites per cmm. averaged only 21 in the Pacific strains and 45 in the Mediterranean strains in patients presumed to have a pristine susceptibility (Young, *et al.* 1947).

The parasites in the patients increased rapidly, attaining the peak parasitemias usually in the second week of the primary attack. These maximum parasitemias averaged about 14,000 per cmm., being higher in the Pacific than in the Mediterranean strains. The highest single parasitemias encountered were 44,200 per cmm. in a Pacific strain and 35,400 per cmm. in a Mediterranean strain.

The maximum temperatures (the highest temperature in a single patient) tended to occur several days earlier than the maximum parasitemias. These peak temperatures averaged 105.0°F. and were accompanied by parasitemias averaging 6,207 per cmm.

The average fever peaks for 934 paroxysms experienced by 79 patients was 104.5°F.

In the tertian type fevers, the average time between fever peaks (periodicity) was 44.4 hours for four Pacific strains and 45.1 hours for one Mediterranean strain. None exhibited a 48-hour periodicity.

Fevers were accompanied by chills 73.2 per cent of the time. The chills were less frequently present with the first five fevers than with the later fevers.

The type of fever at the onset of symptoms was usually quotidian or intermittent, only 8 per cent being tertian. The quotidian and intermittent fevers were readily converted to tertian occurrence by the use of sodium bismuth thioglycollate.

Usually the primary infection produced 10 or more paroxysms. Some patients had experienced as many as 22 paroxysms when the infection was interrupted by drugs.

*Delayed primary attacks in military personnel.* Many troops were infected with malaria under combat conditions but by use of suppressive drugs, a clinical attack after the usual incubation period was prevented. When the suppressive drug was discontinued, however, a clinical attack occurred which is termed "delayed primary attack". Two hundred such patients were studied, the majority of whom had acquired the infections in the Pacific and particularly in New Guinea (Eyles and Young, 1948).

The delayed primary attack occurred on the average 49.1 days after discontinuation of suppressive quinacrine (atabrine).

The parasite-fever threshold was significantly lower at the delayed primary attack than at the subsequent relapse. In 65 patients followed for both, the median value for the delayed primaries was 730 parasites per cmm. against 1,980 parasites per cmm in the subsequent relapse. These parasite fever thresholds are much higher than those for the induced primary attacks in Pacific *vivax* (21 per cmm.) shown previously.

Analyzing the same group of patients, London, *et al.* (1946), found that the delayed primary attack usually was indistinguishable clinically from later relapses. They believe, however, that the parasites and symptoms of the primary attack respond less readily to treatment than do the relapse attacks.

*Response to treatment.* The foreign malarias respond readily to adequate treatment. Of the commonly used drugs, chloroquine is best, quinacrine next, and quinine the poorest (Most, *et al.* 1946).

In relapse attacks, Most, *et al.* (1946), found only 2.1 per cent of 244 patients had fever the day after treatment or subsequently when treated with chloroquine as compared to 8.0 per cent of 391 attacks treated with quinacrine and to 8.7 per cent of 184 attacks treated with quinine. Chloroquine also cleared parasites from the blood stream faster than quinacrine or quinine; the percentages of patients cleared after 24 hours were 38, 26, and 9 respectively. In general, the rate of parasite clearance was grossly related to the initial parasite density.

Gordon, *et al.* (1945), also found that relapsing *vivax* from the Pacific was controlled promptly by quinacrine.

In cases of delayed primary attacks, London, *et al.* (1946), found the fevers and parasitemias less readily controlled by chloroquine than in the relapse attacks.

Treating the primary attacks of induced malaria with chloroquine, we (Young and Eyles, 1948) found only three of 17 patients had fevers 24 hours or later after treatment was started. Even though high parasite densities prevailed, 74 per cent of the parasites were removed in 24 hours and over 99 per cent in 48 hours. One patient was cleared of parasites by the end of 24 hours and all were cleared by the end of five days. There was a positive correlation between the parasite densities and the time to clearance of parasites.

Engstrom, *et al.* (1947), found that patients with primary attacks of induced malaria were cleared of parasites slower with quinacrine than were relapses in soldiers. They relate this to the higher parasite densities in the former.

We (Young and Eyles, 1948) could find no positive correlation between the number of paroxysms experienced in the induced primary attack and the rapidity of clearance of parasites from the blood stream upon treatment.

Sodium bismuth thioglycollate showed a selective action against parasites which were about half grown. An injection of 0.1 gm. of this drug usually removed enough parasites of this age so that that brood failed to cause fevers subsequently. In this, the response is similar to that of the St. Elizabeth strain (Young, *et al.* 1947).

*Relapse rates.* Young, *et al.* (1946), studying a random group of patients where the drug history was unknown or uncertain, found that 40 Mediterranean cases had averaged 7.1 relapses each and 117 Pacific cases had averaged 8.5 relapses each.

Most, *et al.* (1946), found that following treatment with quinine, quinacrine, and chloroquine, the relapse rate at the end of 120 days was 85, 80, and 70 per cent respectively. Other workers (Dieuaide, 1945; Gordon, *et al.* 1946b; Trager, *et al.* 1947) report similar relapse rates after quinacrine and quinine.

A smaller proportion of the Mediterranean malarias seemed to relapse than those from the Pacific. Most, *et al.* (1946), found from 70 to 85 per cent of Pacific *vivax* relapsing against 35 per cent of Mediterranean when treated by the same drugs, where both were observed for 120 days after treatment. Working with induced cases of malaria, Gordon, *et al.* (1946a), found 65 per cent relapses for Pacific and 13 per cent for Mediterranean cases after treatment with quinacrine.

Most relapses occur within 120 days following the last relapse.

The preceding relapse rates were obtained from soldiers having diverse malaria experiences such as length of exposure, different infecting dosages, possibility of infection with different strains, etc.

Craige, *et al.* (1947a), using a known Pacific strain (Chesson) under more controlled conditions showed a correlation with certain factors, viz., the greater the sporozoite inoculum, the shorter the prepatent period, the greater the relapse rate and the shorter the interval between relapses.

A domestic strain of *P. vivax* (St. Elizabeth strain) also shows a high relapse rate after treatment with quinine or quinacrine (Coatney, *et al.* 1948a). There are differ-

ences between this strain and the Pacific strains in relapse patterns. It is probable that the latter may show more relapses per person.

*Relapse intervals.* The intervals between clinical relapses appear to vary according to the drug used, being shortest for quinine, then quinacrine, and longest for chloroquine.

In 292 patients Eyles and Young, (1948), found the median interval to be 59 days (mean 61.6 days). The intervals were measured as from onset of one attack to onset of the next attack. Studying 500 attacks of Pacific and Mediterranean malarias where the interval was measured from completion of treatment to the next attack; Most, *et al.* (1946), found the median intervals to relapse to be: quinine, 24 days; quinacrine, 50 days; and chloroquine, 61 days.

One of the characteristics of the Pacific malarias is the prompt relapse after treatment of either a primary attack or a relapse. In contrast, a domestic strain (St. Elizabeth) when the primary attack or early relapse is treated adequately, shows long latent periods of 6 to 10 months before clinical reactivation. However, when late relapses begin they recur promptly until the end of the infection (Coatney, *et al.* 1948b).

*Parasite density at clinical relapse.* In over 800 clinical relapses, the median parasite density at the fever threshold was 3,200 per cmm. (mean 6,300 per cmm.) (Eyles and Young, 1948). The Mediterranean cases showed a significantly higher parasite fever threshold (median 3,836, mean 7,250) than the Pacific cases (median 2,952, mean 6,030).

No significant variation in parasite level was found between early, middle or late relapses in either the Pacific or Mediterranean group. Also, patients with high or low parasite densities during one clinical relapse tended to have high or low densities, respectively, during a subsequent relapse. Furthermore, those with low parasite densities at one relapse relapsed in similar proportions as those having high parasite densities.

*Parasite patterns.* The activity of the parasites in the blood stream of relapsing patients showed definite patterns in cases where each clinical attack was treated (Eyles and Young, 1948).

The most common type was the appearance of parasites a few days (average 3.5) before the onset of fever which increased in number until fever was produced. This type of parasitemia was called "preclinical asymptomatic parasitemia" and occurred in 77 per cent of the relapse attacks.

About 12 per cent of the cases showed parasitemias between typical clinical attacks, which were designated "interval asymptomatic parasitemias". These parasitemias occurred about 56 days after the onset of the preceding attack, were of low density (270 per cmm.), persisted an average of 12 days, and disappeared without ever provoking typical fevers. The next clinical attack followed about 24 days later.

A third pattern was the appearance of parasitemias after the final clinical attack which were called "terminal asymptomatic parasitemias". These occurred in 25 per cent of the cases. The parasitemias were of a low density and provoked no symptoms. They occurred about 80 days after the last clinical attack and persisted for an average of 44 days, during which time they fluctuated in density, often being

remittent or intermittent. The parasitemias gradually became lower and disappeared.

Both the Pacific and Mediterranean cases were similar in the above patterns.

*Relative prevalence of parasitemias.* A group of 200 patients whose clinical relapses were promptly treated were followed for 120 days to determine the proportion of time that parasites were present in the peripheral blood. The time includes the three types of parasitemias (Eyles and Young, 1948).

During the 120 days, parasites were present in patients with Pacific malaria 16.8 days (13 per cent of the time) of which only 3.9 days (3 per cent of the time) were symptomatic. For the Mediterranean patients, parasites were present 12.9 days (10 per cent) of the 120 days during which only 2.8 days (2.3 per cent of the time) were symptomatic. The difference between the Pacific and Mediterranean cases is probably due to the higher proportion of Pacific cases relapsing.

Seven relapsing patients who did not receive treatment of a clinical relapse were followed to determine parasite behavior. In spite of previous relapses, most of these had extended clinical activity. Terminal asymptomatic parasitemias occurred in all with remittent parasitemias which tended to exhibit lower densities with each recurrence. One patient who had had five relapses previously experienced intermittent clinical relapses for 90 days and on the 300th day of observation still had a low grade parasitemia. The infection in this patient was estimated to be 26 months old at the end of the observation.

In these cases, the proportion of the time of parasitemia was much greater than the 10–13 per cent found in those whose clinical attacks were promptly treated.

*The production of gametocytes.* In induced cases of foreign malarias, gametocytes were found in routine smears (examination of 0.1 cmm. of blood), at the time of first febrile attack in 2.0 per cent of the cases (Eyles and Young, 1948). At that time the number of gametocytes was low and became more prevalent as the disease progressed, reaching fairly high levels during the second week of parasitemia.

In the delayed primary attacks, 22.5 per cent of 200 cases showed gametocytes at the first fever.

In relapses at the time of the first fever, 35.2 per cent of 844 cases from both the Mediterranean and Pacific areas showed gametocytes, being significantly higher in the Mediterranean cases (55.7 per cent) than in the Pacific cases (29.4 per cent). The median density of the Mediterranean cases was 110 male gametocytes per cmm. as compared to a median of 80 per cmm. for the Pacific.

During the symptomatic and asymptomatic parasitemias, similar ratios of gametocytes to total parasites were found. This does not agree with the idea expressed by some that proportionately more gametocytes are produced during the asymptomatic parasitemias (Christenson, *et al.* 1946).

## THE PARASITE IN THE MOSQUITO

*Stage of disease in man and infection in the mosquito.* Mosquitoes were infected by every stage of the disease showing parasites in the blood stream, viz., primary clinical attacks, delayed primary clinical attacks, relapse clinical attacks, and asymptomatic parasite relapses. Patients who had experienced 25 relapses, and others who had had

the disease for 33 months, produced infections in mosquitoes. As long as the patient had an overt malaria infection, the vectors might become infected (Young, *et al.* 1946; Eyles, Young and Burgess, 1948).

The ability of a patient with foreign malaria to infect susceptible mosquitoes seems to be a function of the number of parasites, and consequently the number of gametocytes, present in the peripheral blood stream. This general statement, of course, showed some exceptions. As has been a rather common experience, occasionally patients with low gametocyte counts would infect mosquitoes when others with higher densities would not (Moore, *et al.* 1945; Young, *et al.* 1946). Some patients seem to be better infectors than others. Also, a patient might infect mosquitoes at one time and not at another time, even though the latter instance might seem more favorable. But in the overall picture, higher parasite densities produced more infections in mosquitoes.

In one study, patients showing parasite relapses not accompanied by symptoms infected mosquitoes but at a rate (12 per cent) lower than the parasite relapses accompanied by symptoms (25 per cent) (Eyles, Young, and Burgess, 1948). This is further borne out by a demonstration in the same patient who during an asymptomatic parasitemia infected six per cent of the mosquitos fed upon him as against 87 per cent of the mosquitoes infected when fed during a symptomatic parasitemia.

Furthermore, the higher parasitemias which accompanied the symptoms produced heavier infections (more oocysts per gut) in the mosquitoes than did the lower parasitemias which were not accompanied by fevers.

However, even in the patients whose relapse attacks were treated promptly, the asymptomatic parasitemias were present for longer periods of time (average 12.2 out of a total 120 days) than were the symptomatic parasitemias (average 3.6 days out of 120). In patients whose clinical attacks were not treated, the discrepancy was even greater. Besides, the ill patient was less likely to be exposed to mosquitoes than the asymptomatic patient. Because of these factors, the patients with parsitemias without symptoms might be more likely to spread the disease than those showing symptoms.

*Susceptibility of various species of mosquitoes.* The infectivity of various foreign malarias showing clinical relapses in returned troops to the two important mosquito vectors is shown in Table 1. These data include the 238 cases previously reported (Moore, *et al.* 1945; Young, *et al.* 1946) and additional information obtained subsequent to those reports.

Malarias from widely separated areas infected American anophelines. The overall infection rate for 6,509 *A. quadrimaculatus* was 31.5 per cent and for 4,562 *A. m. freeborni* was 38.0 per cent. Of the 179 lots of *A. quadrimaculatus* fed, 62.4 per cent showed some infected specimens; of 103 lots of *A. m. freeborni*, 73.3 per cent showed infections. As these feedings composed a fairly representative sample, it indicates that if a relapsing patient is exposed to either of the principal vectors, the chances are that about two out of three times he would infect some of the mosquitoes.

It was also shown that the asymptomatic parasitemias would infect *A. quadrimaculatus* (Eyles, Young, and Burgess, 1948). Out of 2,059 mosquitoes, 11.6 per cent were infected. Of the 118 separate lots fed, 28 per cent had infected mosquitoes.

The lower infectivity of the asymptomatic group as compared to the clinical relapsing group is correlated with the lower gametocyte density in the former group.

No evidence was found that the malarias from any one area were significantly more infective to our important vectors than those from another area.

But it was found that the different anopheline mosquitoes, tested under identical conditions, possessed different innate susceptibilities to the foreign malarias (Young, *et al.* 1946; Young and Burgess, 1948).

TABLE 1

*Summary of* A. quadrimaculatus *and* A. m. freeborni *fed upon troops showing clinical relapses of foreign* P. vivax *malaria*

| ORIGIN OF INFECTION | *A. quadrimaculatus* | | | *A. m. freeborni* | | | TOTAL BOTH SPECIES | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mosquito Lots Fed | Individual Mosquitoes | | Mosquito Lots Fed | Individual Mosquitoes | | Mosquito Lots Fed | Individual Mosquitoes | |
| | | Dissected | Per cent Infected | | Dissected | Per cent Infected | | Dissected | Per cent Infected |
| Solomon Islands | 76 | 2,912 | 27.1 | 49 | 2,280 | 38.1 | 125 | 5,192 | 31.9 |
| New Guinea | 53 | 2,008 | 34.0 | 42 | 1,986 | 39.1 | 95 | 3,994 | 36.5 |
| New Britain | — | — | — | 1 | 28 | 60.7 | 1 | 28 | 60.7 |
| Subtotal South Pacific | 129 | 4,920 | 29.9 | 92 | 4,294 | 38.7 | 221 | 9,214 | 34.0 |
| Mediterranean | 41 | 1,306 | 38.9 | 7 | 137 | 26.8 | 48 | 1,443 | 37.0 |
| Liberia | 1 | 47 | 21.3 | 1 | 32 | 40.6 | 2 | 79 | 29.1 |
| C-B-I* | 2 | 89 | 42.7 | 2 | 77 | 35.1 | 4 | 166 | 39.2 |
| Carribbean | 6 | 147 | 13.6 | 1 | 22 | 18.2 | 7 | 169 | 14.2 |
| Total | 179 | 6,509 | 31.5 | 103 | 4,562 | 38.0 | 282** | 11,071 | 34.1 |

* China-Burma-India theater, probably Burma.

** In some cases multiple feedings were made upon one patient, so that 282 feedings were made upon 272 patients.

A comparison of the susceptibility to foreign *vivax* malarias of five anophelines showing the theoretical percentages of infection where the most susceptible species would show a 100 per cent infection has been postulated as follows (Young and Burgess, 1948):

| *Species* | *Theoretical Infective Index* | *Remarks* |
|---|---|---|
| *A. m. freeborni* | 100 | Considered principal vector on West Coast |
| *A. punctipennis* | 86 | |
| *A. quadrimaculatus* | 84 | Considered principal vector in Southern States |
| *A. p. pseudopunctipennis* | 41 | |
| *A. albimanus* | 2 | Suspected as a vector in Lower Rio Grande Valley area |

*A. quadrimaculatus* was used as the standard of comparison. With the exception of *A. punctipennis*, each of the species varied significantly from *A. quadrimaculatus*. *A. m. occidentalis* and *A. p. franciscanus* from the West Coast were compared to *A. m. freeborni* and appeared to have about the same susceptibility, although the numbers tried were not large enough to be significant.

It was further shown that different strains of *A. quadrimaculatus* exhibited a similar susceptibility to the same strain of malaria (Young, *et al.* 1946).

The malaria parasites showed a normal development in the various mosquitoes. In the only cases where abnormal sporozoites were produced, a fungus infection in the mosquitoes seemed to be the cause rather than any inherent mosquito-parasite relationship.

Some of these foreign strains have been maintained through many man-mosquito transfers. Although the mosquito used was different from the vector in the country where the malaria originated, the virulence of the malaria was maintained. This demonstrates the ease with which the *vivax* plasmodium adapted itself to new mosquito hosts.

*Intensity of the infection in mosquitoes.* Data on the number of oocysts per gut, involving both *A. quadrimaculatus* and *A. m. freeborni* which had been infected upon relapsing clinical cases, were tabulated for 1,520 specimens. Half of these had 10 or more oocysts per gut. Ninteteen per cent had 100 or more oocysts per gut, which is a heavy infection. A few of each species of mosquitoes showed extremely heavy infections, having up to 800 oocysts per gut.

The number of sporozoites in the glands for 1,373 specimens, involving both mosquito species, showed 48 per cent with more than 100 sporozoites in the glands and 24 per cent with more than 1,000 sporozoites per gland.

For 239 *A. quadrimaculatus* infected upon patients with asymptomatic parasitemias, the mean number of oocysts per gut was 14.8. This is a lower oocyst density than was found in the mosquitoes which had been fed upon clinical cases.

*Length of the sporogonous cycle in mosquitoes.* The length of the sporogonous cycle is the number of days from the infective feeding to the first appearance of sporozoites in the glands; the latter is presumably the first day that the mosquito is able to transmit the infection.

Kept at about 75°F., this averaged 10.1 days for the *A. m. freeborni* and 10.7 days for the *A. quadrimaculatus*. Further tests will be needed to determine if this difference is significant (Moore, *et al.* 1945; Young, *et al.* 1946).

*Influence of temperature upon infection in mosquitoes.* Some infected lots of mosquitoes, both *A. quadrimaculatus* and *A. m. freeborni*, were divided and half of the mosquitoes kept at fluctuating outside temperatures with the other half at 75°F. The number of infected mosquitoes was about the same when the outside temperatures were high enough to allow development. The only difference noted was a lengthening of the time of the sporogonous cycle when the outside temperatures averaged less than 75°F.

Being incubated at outside temperatures averaging 59°F. for 19 days did not prevent the subsequent development of infections in *A. m. freeborni* when placed in a higher temperature (Moore, *et al.* 1945).

## DISCUSSION

Many of the factors involving the host-parasite relationships between foreign *vivax* malarias and their hosts have been investigated. Where possible the foreign malarias have been compared with each other and with native malarias.

Malarias from the Pacific and Mediterranean areas appeared to be similar in the following points: relative lack of infectivity to Negroes, patterns of parasite relapse and infectivity to native vectors. The Mediterranean malarias had a higher gametocyte density and a higher parasite level at clinical relapse than the Pacific malarias. However, the Pacific malarias showed a higher proportion of patients relapsing after treatment and a greater relative prevalence of parasitemia than did the Mediterranean malarias.

From these criteria, the Pacific malarias might be considered as being more virulent in man than the Mediterranean malarias.

The foreign malarias seem to resemble native malarias on points such as morphology, infectivity to native white population, the course of the disease in the primary attacks, the lack of immunity between strains, the satisfactory response to adequate treatment, the ability to infect our native vectors and to be transmitted by them.

They differ from known domestic strains of *vivax* malaria in the following points, which might be considered as indicating greater virulence of the former: relapse more promptly after treatment of primary attacks, a tendency to produce a greater number of relapses, and possibly shorter prepatent and incubation periods in the human host.

Certain other biological factors of the foreign *vivax* malarias revealed were: None of the infections with tertian occurrences of fevers showed a periodicity of 48 hours but rather one that was shorter; the tendency for patients to show several types of parasitic relapses without being accompanied by symptoms, which resulted in the total time of asymptomatic parasitemias being considerably greater than the time of symptomatic parasitemia; the production of gametocytes in a rather constant ratio to the total number of asexual parasites; the ability of various foreign strains to similarly infect any one species of native mosquito even though the vector was a different species from the vector in the country of origin; the maintenance of virulence after many passages through the new insect vector; the difference in susceptibility of different species of mosquitoes to any one foreign strain; and the ability of the foreign malarias to develop in native mosquitoes under conditions similar to those in nature.

*Epidemiological considerations.* The overall picture of the foreign *vivax* malarias indicates they are as virulent as our native malarias, if not more so. They have shown every prerequisite necessary to establish themselves in this country. What then has been the result of their importation into this country?

It has been estimated that there were about 500,000 hospital admissions for malaria in the Army, most of which were overseas (Simmons, 1947). Although the definite number is not known, many of these relapsed with *vivax* malaria after returning to this country.

According to the morbidity report of Hampton (1946), of the 61,707 cases reported in this country for the year 1945, 19,847 (32 per cent) were of foreign origin. The

latter must have been almost entirely *P. vivax*. Of the 41,671 native cases, there was no breakdown as to species.

An attempt was made to determine the proportion of *P. vivax* in native cases during 1945 by requesting information from Boards of Health in southern states. With the exception of South Carolina, no state had laboratory diagnoses as to species based upon surveys. In South Carolina, four per cent of 1,137 positives found in a survey were *P. vivax* (McDaniel, 1948).

Thus, the data received indicated that the proportion of *P. vivax* in native cases for 1945 might range from four per cent to nearly 100 per cent of the reported cases. So if we arbitrarily assume that about one-half of the native cases reported in 1945 were *P. vivax*, this means that for that year there were about as many foreign cases (19,847) as native cases (20,835) of *P. vivax* present. In other words, the introduction of foreign malarias might be estimated to have doubled the incidence of *vivax* malaria reported for the year 1945.

Faust, *et al.* (1947), estimates that relapsing malaria cases from overseas were dispersed to practically every county in this country. We have shown the native vectors to be good vectors of foreign malarias. Thus, the natural potentialities for the foreign *vivax* strains to become established are great.

Because of the virtual impossibility of distinguishing foreign from native *vivax* malarias, foreign malarias would not be detected by the ordinary blood smears. In areas where there is little malaria now, outbreaks due to importation of foreign strains would be fairly obvious. In fact, such has already been reported (Osgood, 1945). However, the same factors which prevent the occurrence of native malarias in those areas would reduce the likelihood of new strains being established.

The greatest possibility of foreign malarias being spread is in areas such as the South where factors such as abundant vectors, long breeding seasons for mosquitoes, poorer housing, lower economic standards, etc., have allowed the persistence of malaria for many years. And it is in such areas that the spread of foreign malarias, except under unusual and rare circumstances, could not be detected. The result is that probably we shall never be able to determine accurately how much the foreign malarias have and will become established.

But from the knowledge gained of the foreign malarias, we can predict certain occurrences. It is virtually certain that some, if not many, of these foreign strains of *vivax* have and will become established, as every natural requirement is fulfilled. As these foreign strains are immunologically distinct, it means that no protection is gained by previous infections with native malarias. Any one person is susceptible to a potentially greater number of strains of *vivax*, which increases the magnitude of the problem.

It has been a common experience in the past in this and other countries that after wars where troops returned from malarious areas, outbreaks occurred in new areas and often persisted for a number of years. Such has occurred in other countries after World War II (Stevens and Blackman, 1946; Vogt, 1946; Beklemischer, 1946; and Hernberg, 1947).

That such has not been so dramatic here after World War II may be ascribed to various factors, such as improved handling of malaria cases including better treatment, better economic conditions, and better and more extensive malaria control.

Probably the most important factor by far is that these malarias were imported during a time when the malaria rates were declining due to natural or man-made causes (Andrews, 1948).

Should extensive migrations occur before our methods of controlling malaria in man or in nature are universally extended or radically improved through the discovery of even more efficient insecticides or the finding of a truly curative drug, we can expect additional strains to be added to those already present in this country.

*Other results of program.* To test the susceptibility of *A. m. freeborni*, a supposedly important vector, it was necessary to colonize this species on a large scale, which had never been done before. Hardman (1947) developed methods by which it is possible to rear large quantities of this species. As a result it is now possible to study this species of mosquito under experimental conditions.

Methods were developed also which insured the reliable and rapid biting of mosquitoes at a desired time (Burgess and Young, 1944, and unpublished data). This was a very important factor in testing large numbers of mosquitoes against foreign and domestic malarias to evaluate vectors. This was important also in the cooperative programs where a search for better antimalarial drugs is underway as it made possible the mass production of malaria-infected mosquitoes which could be depended upon to infect patients at the desired time (Coatney, *et al.* 1948a).

Furthermore, a strain of South Pacific *vivax* malaria (Chesson) was isolated and has been extensively used in the search for better antimalarial drugs and improved methods of treatment of malaria (Ehrman, *et al.* 1945; Gordon, *et al.* 1946a; Craige, *et al.* 1947b; Shannon, 1945–46; Coatney, *et al.* 1948a).

Foreign malarias were found to be efficacious as an agent in the treatment of neurosyphilis (Young, *et al.* 1947). In fact they have some advantages over indigenous strains as some patients were refractory to the latter because of a previous infection with native malarias.

## SUMMARY AND CONCLUSIONS

1. The program on foreign *Plasmodium vivax* malarias had been carried out by studying the infections in returned troops, by inducing these malarias in other patients, and by feeding large numbers of mosquitoes on malarious patients.

2. The infections in man were quite similar in many respects to domestic malarias but showed a few characteristics which might indicate they are more virulent.

3. Certain factors in the life cycle of the foreign malarias were pointed out, among which was the tendency to show parasitic relapses to a greater extent than clinical relapses.

4. These malarias readily infected our native malaria vectors and were transmitted by them. Malarias from different parts of the world showed similar infectiousness to the same species of domestic vector. Different species of mosquitoes did not show similar susceptibilities to the same strains of foreign malarias. The most dangerous domestic vectors were also the most susceptible to the foreign malarias

5. The foreign malarias in man were infective to mosquitoes during both symptomatic and asymptomatic parasite relapses, being most infective during the former. Gametocytes were produced in a rather constant ratio to the total number of parasites present. Mosquito infections were heavier during clinical attacks but it was

shown that mosquitoes might be infected whenever a sufficient number of parasites were present.

6. The patient with an asymptomatic parasitemia is considered the one most likely to spread the disease.

7. It is estimated that during 1945 there may have been in the United States as many cases of foreign *P. vivax* as there were cases of domestic strains.

8. It is concluded that as a result of the foreign malarias returned by troops, many new strains of *vivax* malaria have been added to those already indigenous to this country and that these new strains will be propagated equally as well. Except in rare instances, there will be no way to distinguish between the native and imported strains in nature.

## REFERENCES


Andrews, J. M. 1948 What's happening to malaria in the U. S. A.? Amer. Jour. Pub. Health (in press).

Beklemischer, V. N. 1946 The influence of migration in malaria during the great patriotic war and the necessary control measures. Med. Parasit. and Parasitic Dis., **15:** 3–24 (abstracted Trop. Dis. Bull., **44:** 639–640, 1947).

Burgess, R. W. and Young, M. D. 1944 Methods of handling and feeding *Anopheles quadrimaculatus* upon malarious patients. Jour. Nat. Mal. Soc., **3:** 241–247.

Coatney, G. R., Cooper, W. C., Young, M. D., Burgess, R. W., and Smarr, R. G. 1947 Studies in human malaria. II. The suppressive action of sulfadiazine and sulfapyrazine against sporozoite-induced *vivax* malaria (St. Elizabeth strain). Amer. Jour. Hyg., **46** (1): 105–118.

Coatney, G. R., Cooper, W. C., and Ruhe, D. S. 1948a Studies in human malaria. VI. The organization of a program for testing antimalarial drugs in prisoner volunteers. Amer. Jour. Hyg., **47:** 113–119.

Coatney, G. R., Cooper, W. C., Ruhe, D. S., Josephson, E. S., Young, M. D., and Burgess, R. W. 1948b Studies in human malaria. VII. The protective and therapeutic action of quinine sulfate against St. Elizabeth strain *vivax* malaria. Amer. Jour. Hyg., **47:** 120–134.

Christenson, H. B., Gordon, H. H., Daniels, W. B., and Lippincott, S. W. 1946 Afebrile parasitemia in imported *vivax* malaria. Amer. Jour. Pub. Health, **36:** 759–761.

Craige, B., Jr., Alving, A. S., Jones, R., Jr., Whorton, C. M., Pullman, T. N., and Eichelberger, L. 1947a The Chesson strain of *Plasmodium vivax* malaria. II. Relationship between prepatent period, latent period and relapse rate. Jour. Inf. Dis., **80:** 228–236.

Craige, B., Jr., Jones, R., Jr., Whorton, C. M., Pullman, T. N., Alving, A. S., and Eichelberger, L. 1947b Clinical standardization of Pamaquin (Plasmochin) in mosquito-induced *vivax* malaria (Chesson strain). Amer. Jour. Trop. Med., **27:** 309–315.

Dieuaide, F. R. 1945 Clinical malaria in wartime. War Med., **7:** 7–11.

Ehrman, F. C., Ellis, J. M., and Young, M. D. 1945 *Plasmodium vivax* Chesson strain. Science, **101** (2624): 377.

Engstrom, W. W., Gordon, H. H., Marble, A., and Brunsting, H. A. 1947 Induced malaria of foreign origin. Arch. Int. Med., **79:** 185–202.

Eyles, D. E. and Young, M. D. 1948 Studies on imported malarias. 7. The parasitological pattern of relapsing *Plasmodium vivax* in military patients. Jour. Nat. Mal. Soc., **7:** 23–37.

Eyles, D. E., Young, M. D., and Burgess, R. W. 1948 Studies on imported malarias. 8. Infectivity to *Anopheles quadrimaculatus* of asymptomatic *Plasmodium vivax* parasitemias. Jour. Nat. Mal. Soc. (in press).

Faust, E. C., Scott, J. A., and McDaniel, G. E. 1947 Malaria mortality and morbidity in the United States for the year 1945. Jour. Nat. Mal. Soc., **6:** 184–191.

Gordon, H. H., Lippincott, S. W., Marble, A., Ball, A. L., Ellerbrook, L. D., and Glass, W. W., Jr. 1945 Clinical features of relapsing *Plasmodium vivax* malaria in soldiers evacuated from the South Pacific area. Arch. Int. Med., **75:** 159–167.

GORDON, H. H., MARBLE, A., ENGSTROM, W. W., BRUNSTING, H. A., AND LIPPINCOTT, S. W. 1946 Relapses following delayed treatment of induced *vivax* malaria of foreign origin. Science, **103:** 391–392.

GORDON, H. H., MARBLE, A., LIPPINCOTT, S. W., HESSELBROOK, W. B., AND ELLERBROOK, L. D. 1946b Clinical and laboratory studies of relapsing *vivax* malaria of Pacific origin. N. Eng. Jour. Med., **234:** 519–523.

HAMPTON, B. C. 1946 Malaria: Numbers of cases reported by the State Health Officers in 1945 as compared with similar data for the years 1939–44. Pub. Health Rep., **61:** 679–683.

HARDMAN, N. F. 1947 Studies on imported malarias. 3. Laboratory rearing of western anophelines. Jour. Nat. Mal. Soc., **6:** 165–172.

HERNBERG, C. A. 1947 The epidemiology of malaria tertiana in Finland during the years 1941–1945. Acta Med. Scandinavica, **127:** 342–360 (abstracted Trop. Dis. Bull., **44:** 951–952, 1947).

LONDON, I. M., KANE, C. A., SCHROEDER, E. F., AND MOST, H. 1946 The delayed primary attack of *vivax* malaria. N. Eng. Jour. Med., **235:** 406–410.

MCDANIEL, G. E. 1948 Personal communication concerning incidence of malaria South Carolina for 1945.

MOORE, J. A., YOUNG, M. D., HARDMAN, N. F., AND STUBBS, T. H. 1945 Studies on imported malarias. 2. Ability of California anophelines to transmit malarias of foreign origin and other considerations. Jour. Nat. Mal. Soc., **4:** 307–329.

MOST, H., LONDON, I. M., KANE, C. A., LAVIETES, P. H., SCHROEDER, E. F., AND HAYMAN, J. M. 1946 Chloroquine for treatment of acute attacks of *vivax* malaria. Jour. Am. Med. Assn., **131:** 963–967.

OSGOOD, S. B. 1945 Malaria and the returning soldier. Jour. Am. Med. Assn., **128:** 512–513.

SHANNON, J. A. 1945–46 The study of antimalarials and antimalarial activity in the human malarias. Harvey Lectures, **41:** 43–89.

SIMMONS, J. S. 1947 Tropical medicine and the challenge of global war. Amer. Jour. Trop. Med., **27:** 1–9.

STEVENS, A. L. AND BLACKMAN, G. C. B. 1946 Benign tertian malaria in England. Brit. Med. Jour., p. 625 (abstracted Trop. Dis. Bull., **44:** 169, 1947).

TRAGER, W., BANG, F. B., AND HAIRSTON, N. G. 1947 The effect of four different therapies on the relapse rate of *vivax* malaria. Amer. Jour. Hyg., **45:** 43–57.

VOGT, E. 1946 A case of malaria in Oslo. Nordisk Med., **32:** 2601–2602 (abstracted Trop. Dis. Bull., **44:** 172, 1947).

WHORTON, C. M., YOUNT, E., JR., JONES, R., JR., ALVING, A. S., PULLMAN, T. N., CRAIGE, B., JR., AND EICHELBERGER, L. 1947 The Chesson strain of *Plasmodium vivax* malaria. III. Clinical aspects. Jour. Inf. Dis., **80:** 237–249.

YOUNG, M. D. AND BURGESS, R. W. 1948 Studies on imported malarias. 9. The comparative susceptibility of *Anopheles quadrimaculatus* and *Anopheles maculipennis freeborni* to foreign *vivax* malaria. Jour. Nat. Mal. Soc. (in press).

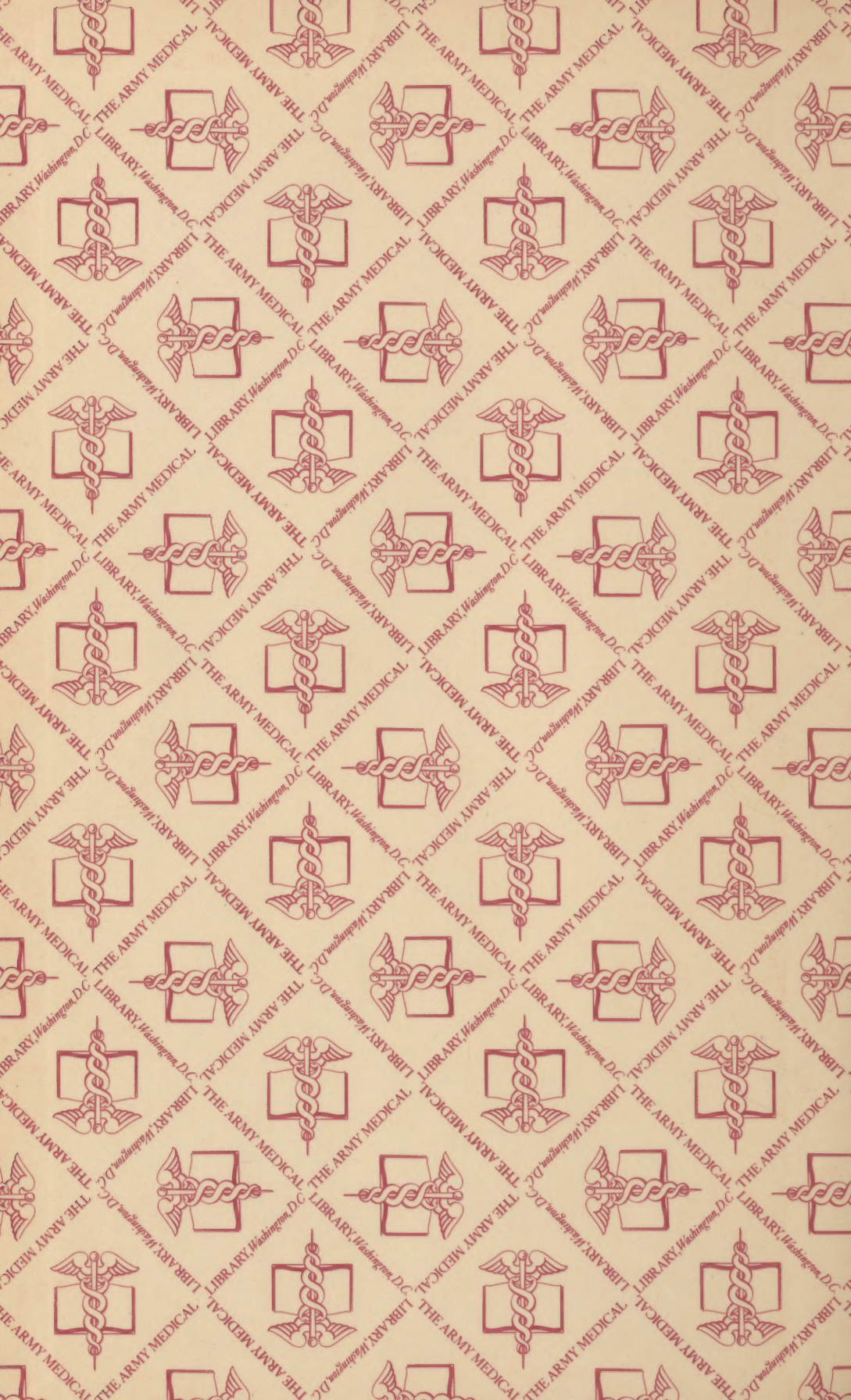
YOUNG, M. D., ELLIS, J. M., AND STUBBS, T. H. 1946 Studies on imported malarias. 5. Transmission of foreign *Plasmodium vivax* by *Anopheles quadrimaculatus*. Amer. Jour. Trop. Med., **26:** 477–482.

YOUNG, M. D., ELLIS, J. M., AND STUBBS, T. H. 1947 Studies on imported malarias. 6. Some characteristics of foreign *vivax* malaria induced in neurosyphilitic patients. Amer. Jour. Trop. Med., **27:** 585–596.

YOUNG, M. D. AND EYLES, D. E. 1948 The efficacy of chloroquine, quinacrine, quinine, and totaquine in the treatment of *Plasmodium malariae* infections (quartan malaria). Amer. Jour. Trop. Med., **28:** 23–28.

YOUNG, M. D., STUBBS, T. H., ELLIS, J. M., BURGESS, R. W., AND EYLES, D. E. 1946 Studies on imported malarias. 4. The infectivity of malarias of foreign origin to anophelines of the Southern United States. Amer. Jour. Hyg., **43:** 326–341.

YOUNG, M. D., STUBBS, T. H., MOORE, J. A., EHRMAN, F. C., HARDMAN, N. F., ELLIS, J. M., AND BURGESS, R. W. 1945 Studies on imported malarias. 1. Ability of domestic mosquitoes to transmit *vivax* malaria of foreign origin. Jour. Nat. Mal. Soc., **4:** 127–131.


THE ARMY MEDICAL
LIBRARY, Washington, D.C.